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Epilepsy and pregnancy: Report of an Epilepsy Research Foundation Workshop

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Abstract

Pregnancy in women with epilepsy (WWE) is known to be associated with a higher risk of congenital malformations than is associated with pregnancy in non-epileptic women. Several factors have been identified to account for the increased risk, including the direct teratogenic effects of antiepileptic drug (AED) therapy, indirect effects of these drugs by interfering with folate metabolism, genetic abnormalities in drug or folate metabolism, and possibly an arrhythmogenic effect of maternal drug therapy on the embryonic heart, leading to ischaemia in developing tissues. A harmful effect of maternal seizures on the developing embryo has not been proven, although seizures and status epilepticus account for most of the excess maternal mortality in women with epilepsy. Abrupt withdrawal of drug therapy by the mother may be an important contributory factor. Less is known about the psychomotor development of children born to mothers with epilepsy because few studies have been designed to follow their progress throughout childhood. Retrospective studies suggest that impaired cognitive development may be associated with maternal drug therapy, particularly valproate. There is an urgent need to evaluate these risks and, with this in mind, several prospective registers have been set up to collect data from pregnancies in women with epilepsy.

Keywords: Antiepileptic drugs; Pregnancy; Teratogenicity; Congenital malformations; Epilepsy

1. Introduction

On the 16th and 17th of September 2002 the Epilepsy Research Foundation held a workshop in Worcester College, Oxford. The purpose of the workshop was to discuss some of the issues faced by women with epilepsy (WWE) who wish to become pregnant. The outcome of pregnancies to WWE is generally recognized to be two to three times poorer than that of the general population. Additional risk factors faced by the mother and her child include potential teratogenic actions of antiepileptic drugs (AEDs) taking during pregnancy, the possible effect of maternal seizures on the developing foetus, and genetic risks, which may carry over from the mother to her child. Although considerable advances have been made in our understanding of these issues, particularly

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with the advent of molecular biological techniques in research, there are still many questions, which are unanswered.

The Foundation felt there was a need for the workshop in order to bring together international experts in the field to review current research and to discuss methods for collecting data in the future.

The workshop was attended by 22 invited participants from Austria, Canada, Denmark, Finland, Germany, Italy, Netherlands, Sweden, USA, and UK, 21 invited observers from Research Units and Industry and six Members of Epilepsy Research Foundation.

2. Congenital malformations

Around one in 200 pregnancies are thought to be exposed to AEDs. Since the late 1960s, studies looking at the malformation rates in children who have been exposed to AEDs during pregnancy consistently revealed a higher percentage of malformations in these children than in those who had not been exposed.

It has also been shown that children exposed to more than one type of drug during pregnancy have an even greater risk of malformations being present at birth. Congenital malformations associated with AED exposure during pregnancy present as a wide range of anomalies affecting many different body systems including the central nervous system, the gastrointestinal tract and the cardiovascular system. One malformation, which has been of particular concern, is that which affects the development of the neural tube. During foetal development the initially flat neural plate folds to form a tube that seals at the top. The processes involved in the closing of the neural tube have been shown to involve folic acid, and three commonly used AED's, carbamazepine (CBZ), phenytoin (PHT) and sodium valproate, are known to interfere with folic acid metabolism. Valproate in particular has been associated with a 1–2% risk of spina bifida. As well as the obvious malformations there are a number of other deformities seen which vary in severity, including a typical craniofacial appearance with widespread eyes, flat nasal bridge and small upturned nose, underdeveloped fingernails and neurodevelopmental delay.

The mechanisms behind these malformations are on the whole poorly understood, but are thought to involve a number of factors including drug metabolism by the mother and foetus, drug interference with folate metabolism, maternal seizures and maternal genes.

It is important to be able to elucidate the mechanisms behind AED teratogenesis to allow for the development of new drugs which are as efficacious at controlling seizures as the parent compound but pose less risk to the developing foetus.

2.1. Drugs and malformations[1]

AED's have been used to treat epilepsy for over 80 years. In the UK, the first suspicions that AED's might be teratogenic were raised by Meadow writing in the Lancet in 1968 when he reported a group of children who had been born with a variety of birth defects following in utero exposure to phenobarbital, PHT or troxidone (Meadow, 1968). Other anecdotal reports followed and by the early 1970s it was accepted that the rate of malformations in the children of mothers with epilepsy was about double that in the general population.

Proven or suggested teratogenic outcomes range from major congenital malformations (MCM), syndromes of dysmorphism and intra-uterine death, to abnormal physiological adaptation in the newborn period and developmental delay or specific cognitive defects discovered from birth until adulthood. Third generation (F3) reproductive toxicity has hardly been studied (Dessens et al., 2001), but recent findings showing an increased frequency of hypospadias in the offspring of daughters prenatally exposed to diethylstilboestrol (Klip et al., 2002) suggests that this endpoint should also be included in the list of possible

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outcomes of reproductive studies involving maternal AED use.

The first generation of AEDs, phenobarbital (PB) and PHT, are mainly associated with heart defects and facial clefts. The second generation AEDs, valproate and CBZ, are primarily associated with spina bifida aperta (valproate), neural tube defects (NTDs) (CBZ), hypospadias (both), radial aplasia (rare but valproate specific), and probably an autism-like disorder (valproate).

The major shift in type of malformations associated with AEDs from the first versus the second generation supports the causality of the association. Dose–effect relations have been observed for valproate and for PB. This also supports the causality of the association between exposure and outcome (Samren et al., 1999). The third generation of new AEDs consists of a large number of structurally related or different compounds, some of which have teratogenic activity in preclinical animal experiments, but none have sufficient human pregnancy experience to assess their safety or teratogenicity when used by pregnant women with epilepsy.

Periconceptional folic acid supplementation at low dose or high dose has not been investigated adequately for prophylactic effects or side effects to be evaluated in cases of maternal AED use (Berg and Lindhout, 2002). Animal experiments testing folinic acid as an antidote for valproate induced exencephaly suggested a partial protective effect of the supplementation, but could not be reproduced by other groups. Other studies suggest that pantothenic acid (vitamin B5) is effective as an antidote, which was not explored further until to date.

Most AEDs are metabolized and give rise to a large number of different metabolites. In fact, the adult patient and the embryo or foetus is exposed to a mixture of parent compound and a varying number of metabolites. Imbalance between uptake or formation and degradation or excretion of toxic compounds, whether parent compound or metabolite, may influence the teratogenic effects in combinations. Metabolic interactions between AEDs and with other environmental factors like social drugs and food constituents may alter, increase or decrease the exposure to noxious substances. Also pharmacogenetic factors may disturb this balance (Lindhout, 1992). Interactions and genetic variation may also be relevant at the pharmacodynamic level, the embryonic molecular targets. Combinations of CBZ + valproate + PB ± PHT, or PB + primidone + PHT, or benzodiazepine polytherapy are associated with higher than average risk. It remains to be determined at which level the interactions leading to teratogenic outcome play a role.

Interspecies differences in susceptibility to teratogens limit the predictive value of preclinical reproductive toxicity testing in animals. Past experience, namely that almost all AEDs are teratogenic, necessitates the conduct of population-based studies to learn from human experience. Currently available AEDs are fully effective and without side effects in only a small minority of patients. For this reason, new compounds are being developed and marketed, more than ever before. Most new drugs are first applied in an add-on regime. This implies polytherapy, which increases the problem of limited predictability of preclinical reproductive toxicity test results of single compounds. There is no regulatory rule that new compounds should be tested for co-teratogenicity with other commonly used AEDs.

New ways to improve insight into teratogenic indicators and mechanisms may include the development of animal models mimicking the currently known human teratogenic syndromes. It is especially important to investigate the temporo–spatial embryonic and foetal gene expression patterns and to compare susceptible and resistant strains, exposure to single compounds and combinations, and effects of potential antidotes.

Prospective population-based studies clearly play an important role in post-marketing evaluation of AEDs safety in human pregnancies. The frequency of maternal epilepsy is 1/200 and of chronic AED treatment during pregnancy 4/5. The additional risk of foetal malformations associated with AEDs exposure is 5/100. This results in a population frequency of newborn babies with one or more malformation due to maternal AED use of 20/100 000. This is in the same range as the prevalence of phenylketonuria or congenital hy-

pothyroidism at birth, disorders which are targets for national screening programs.

In contrast to these screening programs, funding of prospective drug studies is heavily dependent on sponsoring by drug manufacturers. Many interested and capable clinical investigators may have conflicts of interests due to involvement in different clinical studies, trials or other relations. These issues have to be addressed openly at all levels in the organization, and should be part of a protocol, in order to support the investigators in maintaining their independence, freedom of publication, and validity of the results, to the benefit of our patients.

2.2. Patterns of malformations[2]

In early reports of birth defects following in utero exposure to AEDs, no specific pattern of problems was noted in the affected children. However, Hanson and Smith (1975) noted similarities between children who had been exposed to PHT in utero. These children were often growth retarded with a small head circumference, and dysmorphic facies. As a group they had an increased incidence of oro-facial clefts and cardiac defects and a very characteristic feature was the presence of distal digital hypoplasia with small nails. This pattern of problems became known as the Foetal Phenytoin Syndrome. In addition to the features mentioned there have also been some rare reports of holoprosencephaly and a chondrodysplasia punctata-like syndrome with flat facies and stippled epiphyses occurring after PHT exposure in utero.

A Foetal Valproate Syndrome was recognized by Di Liberti et al. (1984) The facies were reported to be very distinctive with a ridged metopic suture leading to trigonocephaly, short, anteverted nose, broad nasal bridge and small mouth with thin upper lip and everted lower lip. The eyebrows are often neatly arched and deficient at the inner aspects. Valproate exposure is associated with a variety of malformations including NTDs, cleft palate (CL(P)), radial ray defects, cardiac anomalies and ophthalmological problems. Minor malformations of the limbs such as overlapping toes and joint laxity are common findings and affected children often have a clumsy gait and poor lower limb musculature. The Foetal Valproate Syndrome also comprises learning and behavioral abnormalities. Withdrawal symptoms with jitteriness and hypoglycaemia may be present at birth and developmental milestones are often delayed, particularly in the area of speech. Toilet training appears to be a particular problem. This group of children appears to have a higher incidence than normal of learning difficulties.

A pattern of abnormalities has also been identified in children exposed to CBZ in utero, although these are much subtler than with exposure to valproate. CBZ exposed children may have minor dysmorphic features (DF) with a short nose, broad nasal bridge and small chin.

Nail hypoplasia is sometimes seen and there appears to be an increased incidence of spina bifida and cardiac anomalies. There have been some reported learning difficulties in association with CBZ exposure. At the present time we do not have enough information about the newer drugs to determine whether they are associated with specific patterns of malformations and developmental problems. It is clear that polytherapy, however, is more likely to have an adverse effect on the foetus.

Although a lot of attention has been drawn to the DF seen in some children exposed to AEDs in utero, it is important to take into account both normal variation and the family features when assessing these. A careful history must also be taken to make sure that the child has not been exposed to other teratogens e.g. alcohol or maternal diabetes. The facial features of the 'Foetal Anticonvulsant Syndromes (FAS)' change and evolve over time, and when assessing an older child it is often useful to look at earlier photographs, too, when the dysmorphic facial features are often much more apparent.

There is some debate as to whether identifying the dysmorphic facial features associated with FAS serves a useful purpose. The dysmorphologist would argue that both parents and child would

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benefit from having a diagnosis for their child's problems, and that this could influence management of the child, prevent complications arising and enable the family to access sources of support. At the present time it is not clear by what mechanism AED exposure causes patterns of malformations. Whilst some features may arise due to a general toxic effect on the embryo causing increased cell death, other more specific malformations may be caused by interference in specific gene pathways. Further study of the FAS may help us to clarify these further, and identify ways of lessening risk.

2.3. Mechanisms

2.3.1. Drugs

2.3.1.1. Toxic drugs and metabolites[3]. Developmental pathologies may result from endogenous or xenobiotic-enhanced formation of reactive oxygen species (ROS), which oxidatively damage cellular macromolecules and/or alter signal transduction. Studies in Toronto have used as models several drugs (PHT and related anticonvulsant drugs, thalidomide), environmental chemicals (benzo[a]pyrene) (B[a]P) and ionizing irradiation (IR) to examine this hypothesis in vivo and in embryo and cell culture using mouse, rat and rabbit models.

These chemical teratogens themselves are relatively nontoxic, and require enzymatic bioactivation to form a highly toxic reactive intermediary metabolite. Drug bioactivation and ROS formation likely occur within the embryo rather than in maternal tissues, since reactive intermediates are highly unstable. Nevertheless, at least for PHT and B[a]P, reduced maternal cytochromes P450 or glucuronidation can result in reduced maternal elimination and increased transfer to the embryo of B[a]P, or PHT and its major hydroxylated metabolite (HPPH), the latter two of which are equipotent in causing oxidative chromosomal damage and embryopathies (Wells and Winn, 1996).

In the embryo, in contrast to the low or negligible activities of P450s, embryonic prostaglandin H synthases (PHSs) and lipoxygenases (LPOs) are highly expressed, and these enzymes can bioactivate xenobiotics to free radical intermediates that initiate ROS formation, resulting in oxidation of proteins, glutathione (GSH), lipids and DNA, the latter including formation of an 8-oxoguanine (8-oxoG) lesion with toxic potential (Wells and Winn, 1996; Parman et al., 1998; Parman and Wells, 2002). PHS substrates include PHT, HPPH, structurally related anticonvulsant drugs (mephenytoin, trimethadione (TMD), PB) and/or their *N*-demethylated metabolites (nirvanol, dimethadione), the sedative/antileprotic drug thalidomide and the environmental chemical B[a]P. ROS also may be produced by redox cycling of quinone drug metabolites, although this may be limited to cases where deficient maternal glucuronidation results in transfer to the embryo of high concentrations of di-hydroxylated metabolites. Shanks et al. (1989) have provided evidence that ROS alternatively could be formed via reperfusion following inhibition of the embryonic heart rate, which we first demonstrated for PHT at therapeutic concentrations. However, it is difficult to assess the embryopathic importance of this latter mechanism. In studies carried out in Toronto, a maximally reduced heart rate occurred at a low and relatively non-embryopathic concentration of PHT, and in later studies, embryopathies produced in culture were similar whether PHT was washed out after 4 h, or maintained throughout the culture period, precluding reperfusion effects. Also, not all ROS-initiating teratogens (e.g. B[a]P, thalidomide) necessarily inhibit embryonic heart rate, nor is it clear whether the embryopathic isomers of racemic mephenytoin and nirvanol stereoselectively inhibit heart rate. On the other hand, PHT, HPPH and B[a]P initiate DNA oxidation and chromosomal damage in cell culture, precluding a dependence upon cardiovascular

[3] Professor Peter G Wells, Faculty of Pharmacy and Department of Pharmacology, University of Toronto, Toronto, Ont., Canada.

alterations. More directly, xenobiotic-initiated DNA oxidation and embryopathies are reduced in PHS knockout mice, and in mice treated with PHS and/or LPO inhibitors, suggesting that PHS-catalyzed bioactivation may play an important role in drug-initiated ROS formation.

The embryopathic importance of ROS is suggested by several lines of evidence, including the observation that thalidomide causes embryonic DNA oxidation in susceptible (rabbit) but not resistant (mice) species (Parman et al., 1999). Similarly, antioxidative enzymes (superoxide dismutase, catalase), antioxidants (glutathione [GSH], caffeic acid, vitamin E), iron chelators (desferrioxamine) and free radical trapping agents (phenylbutylnitrone) reduce the embryopathic effects of ROS-initiating teratogens (Wells and Winn, 1996; Winn and Wells, 1999; Parman et al., 1999). Conversely, embryopathies are increased in mutant mice deficient in the antioxidative enzyme glucose-6-phosphate dehydrogenase (G6PD; Nicol et al., 2000), or by GSH depletion, or inhibition of GSH peroxidase or GSH reductase (Wells and Winn, 1996). In biochemically compromised animals, even endogenous ROS formed during normal metabolic activity can be toxic, as evidenced by increased embryopathies in untreated G6PD-deficient and ATM-deficient mice. Inducible nitric oxide synthase knockout mice are partially protected, suggesting that reactive nitrogen species contribute, although the molecular mechanism in this case is not clear. During organogenesis, the potent embryopathic effects of IR, which cannot react with receptors or form adducts, also are consistent with a ROS-dependent embryopathic mechanism.

Since ROS oxidize GSH and all cellular macromolecules including DNA, as well as reversibly activate a number of signal transduction pathways, it is important to determine which effects contribute causally to embryopathic outcomes. For DNA oxidation, in addition to mutational consequences, the 8-oxoG lesion can result in a deficient rate and fidelity of transcription, which could be embryopathic. To estimate the embryopathic contribution of oxidative DNA damage by endogenous and teratogen-enhanced ROS, knockout mice deficient in DNA damage recognition and repairs, have been used in Toronto. In normal outbred mice in vivo, PHT-initiated formation of 8-oxoG in the embryo is repaired within 24 h, similar to the rate in maternal tissues (Wells and Winn, 1996), suggesting high embryonic repair activity. With ATM-deficient mice, even untreated embryos had increased embryopathies, providing further evidence of a toxic potential for endogenous oxidative stress. Both p53-deficient (Wells and Winn, 1996) and ATM-deficient knockout mice were more susceptible to the embryopathic effects of IR, PHT and/or B[a]P, suggesting at least for these ROS-initiating teratogens the importance of DNA, as distinct from other cellular macromolecules, as a molecular target mediating embryopathies, and deficiencies in DNA repair activity as a risk factor. Heterozygotes showed intermediate susceptibility to both endogenous and xenobiotic-enhanced ROS, indicating that DNA repair may constitute an important risk factor.

The alternative possible contribution of ROS via reversibly activating signal transduction pathways was investigated with respect to the Ras pathway (Winn and Wells, 2002) and its downstream NF-kB transducing protein. Both proteins were constitutively expressed in the embryo, and expression was enhanced by PHT. Inhibition of either the Ras pathway using a farnesyl–protein transferase inhibitor, or the NF-kB pathway using an antisense oligonucleotide, resulted in protection, implicating ROS-mediated signal transduction in the embryopathic mechanism.

Thus, embryonic PHS/LPO-catalyzed bioactivation of such teratogens to free radical intermediates, as distinct from receptor-mediated mechanisms or the P450-dependent formation of covalent drug-macromolecular adducts, may play a fundamental role in their embryopathic mechanism. Furthermore, the balance among maternal drug elimination and embryonic pathways for teratogen bioactivation, ROS detoxification, oxidative DNA damage and repair, and ROS-mediated signal transduction may constitute an important determinant of teratological risk. If similar mechanisms are involved in humans, then embryos with an unfavorable biochemical balance among these pathways may experience embryo-

pathic effects even at therapeutic drug concentrations.

2.3.2. Discussion[4]

Professor Pirmohamed opened the discussion by saying that although every drug undergoing development has to be tested in two animal species for its teratogenic potential for drug regulatory purposes, this does not necessarily exclude its potential to cause teratogenic malformations in man. This usually results in the insertion of a generic statement in the product information warning against the use of the drug in pregnancy. This has been highlighted in a recent study which showed that over 90% of drugs approved by the FDA between 1980 and 2000 did not provide adequate information on the risks of drug use in pregnancy (Lo and Friedman, 2002). This is also true of AEDs; there are many older drugs that are known to have a teratogenic potential, while many of the newer compounds are of unknown risk.

A reason for the lack of information and adequate assessment of risk is largely due to our ignorance of the possible mechanisms of teratogenicity. It is likely that there are multiple mechanisms involved even with the same drug. An enduring hypothesis that has been postulated for many drugs, including anticonvulsants, is that of metabolism of the drugs to toxic metabolites, a process that can be termed bioactivation. Indeed, many of the anticonvulsants have been shown to undergo bioactivation to toxic metabolites such as arene oxides, quinones, N-hydroxy compounds, and free radicals. The studies performed by Wells and co-workers in particular clearly show the potential importance of free radical species in the pathogenesis of teratogenicity (Wells et al., 1997). Free radicals are hard electrophiles and as such are more likely than softer electrophiles to bind to DNA with the potential to produce embryopathy.

However, it is important to note that evidence implicating toxic metabolites (by necessity) is largely generated from animal studies, which vary in their susceptibility to malformations compared with man. Specific aspects that should borne in mind include:

i) Different animal species vary considerably in their sensitivity to thalidomide, with rabbits being more sensitive than rats.
ii) Certain drugs such as aspirin can cause defects in animals but do not cause teratogenicity in man, and vice versa for drugs such as misoprostol.
iii) Teratogenicity studies in animals are performed with single drugs, yet the clinical evidence suggests that polytherapy is more important with respect to anticonvulsant teratogenicity.
iv) The use of transgenic knockouts or chemical inhibitors to implicate specific pathways in the mechanisms of teratogenicity may also be too simplistic since they ignore possible compensatory changes that may occur in response to the absence of that specific physiological pathway.

Thus, in interpreting data from animal studies, it is always important to consider whether the findings can be extrapolated to man.

In order for a toxic metabolite to cause teratogenicity, it must be formed either in the mother and transported across the placenta or within the foetus after passage of the parent drug across the placenta. Given the highly reactive nature of these chemical species and their short half-lives, it seems unlikely that toxic metabolites will be transported across the placenta. Formation within the foetus, therefore, requires the presence of functional drug metabolizing enzymes. Indeed, the evidence for this is now emerging; studies particularly in foetal liver have shown the presence of both phase I (e.g. cytochrome P450 isoforms) and phase II (e.g. glucuronyl transferases) enzymes, as well as enzymes such as prostaglandin synthase and LPO, implicated in recent studies on free radical-mediated teratogenicity. However, whether these enzymes are present in all the organs affected by toxicity is not clear at present.

The formation of toxic metabolites in the foetus needs to be counter-balanced by protective detox-

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ification or bioinactivation processes. In this respect, glutathione and the various enzymes involved in the glutathione cycle are going to be important. This is an important area that does need a great deal of research. The picture is complicated by the fact that measurement of total cellular content of glutathione in the foetus, and the effect of toxic metabolite exposure on total glutathione level, may be too simplistic and will not provide the complete picture. This has been highlighted by recent studies with thalidomide showing preferential modulation of the nuclear redox status in sensitive but not in insensitive species (Hansen et al., 2002) If one accepts that toxic metabolites are important in the pathogenesis of teratogenicity, then the crucial factor in determining toxicity is going to be the balance between bioactivation of the drug to free radical species and other reactive metabolites and their bioinactivation to harmless metabolites. There are, however, many questions that need to be answered in respect of this balance, including:

i) Does this balance between bioactivation and bioinactivation vary between different stages of pregnancy?
ii) Does the balance vary between different individuals?
iii) Is the variation in-balance a determinant of individual susceptibility to teratogenic malformations? This is important to elucidate given that there is evidence of genetic predisposition to anticonvulsant teratogenicity, and may allow the development of pharmacogenetic strategies for prevention of malformations.
iv) If the balance is in favor of bioactivation, can it be improved through the use of antioxidants, thereby allowing bioinactivation processes to become predominant?

Preliminary studies in animal models certainly support this concept.

Finally, it is important to acknowledge two important facts when considering the role of toxic metabolites in anticonvulsant teratogenicity. First, we do not have a complete knowledge of the oxidant and antioxidant processes that normally operate in the body even in adults, let alone in the foetus. Undoubtedly, many important cellular protective mechanisms will be identified in the future. The availability of novel genomic and proteomic technologies may allow the definition of known and novel pathways, but their success is going to be predicated by the availability of accurately phenotyped foetal tissue. Second, the pathogenesis of anticonvulsant teratogenicity is likely to be multi-factorial involving processes other than the formation of toxic metabolites. The contribution of each mechanism to the overall clinical picture will require a co-ordinated research effort.

In general discussion, Professor Wells commented that the mechanisms of drug teratogenesis are very complex with a multitude of factors effecting outcome, and that due to the multiple effects toxins can have on cellular function, large numbers of experiments will be needed to fully understand the principles behind teratogenesis. Professor Lindhout suggested that one way to try and look at the mechanisms behind the drug teratogenesis may be to look first at human studies, then at animal models presenting with the same phenotypes, then to sensitive and insensitive strains of these species and ultimately to genomics to try and see where the differences lay. It was generally acknowledged that there were problems with looking to animal models to answer these questions as different species have diverse metabolic processes which means that potential teratogenic outcomes may be difficult to extrapolated to humans. Professor Lindhout further commented that the advent of proteomics and genomics would potentially be very helpful in trying to determine risk factors associated with AEDs, and it could be worthwhile sending families for genetic analysis and collecting blood samples for future examination.

2.3.2.1. Valproate[5]. Valproate (valproic acid) is increasingly used for other purposes than the treatment of epilepsy: this drug has been found

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valuable for prophylaxis of migraine headaches, and the treatment of bipolar disorders. More recently it has also been proposed for cancer treatment, in particular for the treatment of malignancies of the central nervous system (Blaheta et al., 2002). However, a number of unwanted effects are apparent, including teratogenesis, liver toxicity and weight gain (Nau et al., 1991).

The molecular mechanisms which may be at the basis of some of the wanted and unwanted effects have been reviewed by Lampen et al. (1999, 2001), Werling et al. (2001). From the viewpoint of structure–activity relationships and effects on gene expression it is argued that that there is some relation between the mechanisms involved in valproate-induced teratogenesis, anti-cancer action and possibly also neuroprotection.

A number of new compounds were developed experimentally which are structurally related to valproate (Andrews et al., 1997; Bojic et al., 1998; Ehlers et al., 1992; Hauck et al., 1992; Fig. 1). On the one hand, compounds with low potency for induction of NTDs in the mouse model were developed which still exert the desired anticonvulsant effect. On the other hand, potent teratogens were developed in order to study the mechanisms of the teratogenic action of this class of compounds. The activation of the nuclear receptor PPARδ by valproate appears to be very important in determining its teratogenicity because it correlates closely with the potency of these carboxylic acids in the in vivo mouse model of exencephaly induction (Lampen et al., 1999, 2001; Werling et al., 2001). Preliminary results indicate a possible involvement of adhesion molecules, in particular NCAM-PSA, in the teratogenic and anti-cancer action of valproate. The study of molecular mechanisms is valuable for the development of new compounds with the desired activities, but lacking the unwanted effects.

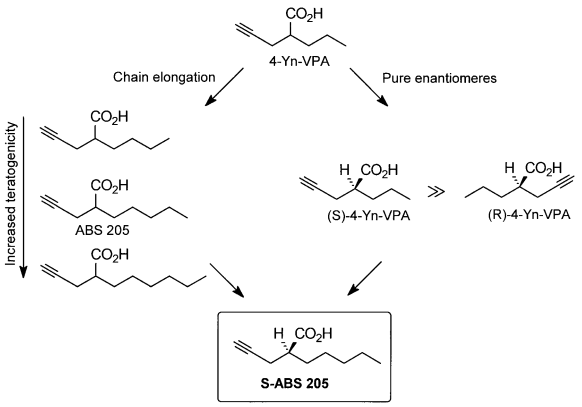

Fig. 1.

The valproate analogue 4-yn-VPA is more teratogenic than the parent drug in the exencephaly mouse model. Increasing the saturated chain of this molecule considerably increases the teratogenic potency (pentyl-4-yn-VPA, ABS 205). The pure enantiomers of the 4-yn-VPA have drastically different teratogenic potencies with the S-enantiomer being much more potent than the R-enantiomer. The S-enantiomer of pentyl-4-yn VPA (S-ABS 202) is the most potent teratogen synthesized up to now within this series of carboxylic acids.

2.3.3. *Discussion*[6]

Dr Clayton-Smith pointed out that from a therapeutic point of view, sodium valproate (VPA) is a widely used drug due to its wide range of anticonvulsant effects and relatively mild side effects. In contrast to the teratogenic effects of some of the other AEDs, valproate produces some very specific birth defects such as spina bifida, radial ray defects, CL(P) and myopia, suggesting that it may act in specific developmental pathways. Animal experiments have shown that the timing of exposure of an embryo or foetus to valproic acid may determine the type of malformation seen. Effects on the major organ systems are expected with first trimester exposure. More recently there has been concern about a possible increased risk of neurodevelopmental problems in children with valproate exposure and neurodevelopmental effects are more likely to be due to exposure later in the pregnancy, when brain cells are proliferating and migrating. It is suggested that valproate has many actions, from an effect on ion channels to anti-tumor activity. There are several mechanisms by which valproate could have a teratogenic effect.

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It could have a direct toxic effect on the embryo causing an increase of programmed cell death, similar to the effect of alcohol exposure. This is supported by the fact that valproate has been shown to accumulate at the neuroepithelium. Further mechanisms for teratogenicity include alteration of folic acid metabolism and alteration of expression of genes in specific developmental pathways. Work by Faiella et al. (2000) has suggested that interference with foetal hox genes in rodent embryos might account for the excess of limb defects seen with valproate exposure. Nau described a further possible mechanism, which involves interference with one of the genetic pathways involving neuronal cell adhesion molecules. This type of pathway has already been implicated in human disease and may be relevant to the neurodevelopmental effects seen in some exposed children. Finally, other presenters have proposed that there may be an effect on the foetal heart, predisposing to arrhythmias, which interfere with the circulation and causing, hypoxia and acidosis. All of these possibilities will need further investigation, however.

It has been demonstrated that the various properties of VPA can be altered by manipulation of the chemical structure of the various side arms. It may be that work in this area may help to determine if there is a derivative of VPA, which is less teratogenic. Whatever the mechanisms concerned, there appears to be a genetic component to valproate susceptibility, with some foetuses being more susceptible to teratogenic effects even at relatively low doses. The question is as to whether genetically susceptible mothers or foetuses can be identified.

In the general discussion Professor Nau spoke about the possibilities of developing new compounds, again he highlighted the need to know the mechanisms behind the teratogenic effect before we can try and develop drugs which are as efficacious at controlling the seizures but have lower risks associated with them. To address the issue of being able to identify those at risk, Professor Finnell spoke of his studies on inbred mice strains, which have a high degree of genetic susceptibility and resistance. They are well on the way to having completed a whole genome scan and identifying the critical region. The completion of this could lead to a diagnostic tool.

2.3.3.1. Drug induced embryonic arrhythmia[7]. The AEDs PHT, PB, TMD and CBZ cause a similar pattern of foetal adverse effects. This is characterized by impaired intrauterine growth, minor abnormalities related to retarded growth and major structural defects, such as cardiovascular malformations, orofacial clefts and digital reductions (Azarbayjani and Danielsson, 1998). Polytherapy is associated with higher risk, and also more severe malformations. There is accumulating evidence suggesting that their teratogenicity is caused by a common mechanism namely concentration-dependent embryonic bradycardia and arrhythmia/cardiac arrest due to the ability of PHT, PB, TMD and CBZ to block the rapid component of the delayed rectifying K ion current (Ikr). The arrhythmia causes periods of interrupted oxygen supply and generation of highly toxic ROS in the embryonic tissues during the reoxygenation/reperfusion phase (Azarbayjani and Danielsson, 1998; Danielsson et al., 2001).

This mechanism is supported by experimental studies showing (Azarbayjani and Danielsson, 1998; Danielsson et al., 2001; Azarbayjani and Danielsson, 2001, 2002; Sköld et al., 2002),

i) periods of interrupted oxygen supply to the embryo cause similar stage-specific malformations (preceded by haemorrhage and necrosis) as single doses of PHT;
ii) the non-innervated embryonic heart is dependent on Ikr for regulation of heart rhythm during a restricted period and is very susceptible to arrhythmogenic action by Ikr blockers;

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iii) AEDs like valproate or vigabatrin, which not block Ikr, do not have any potential to cause embryonic arrhythmia or hypoxia-related malformations;
iv) Ikr blockers like dofetilide and cisapride (not used as AEDs) cause embryonic arrhythmia, embryonic hypoxia and similar malformations as PHT, PB, TMD and CBZ;
v) simultaneous exposure to more than one AED (PHT, PB, TMD or CBZ) results in more severe embryonic cardiac adverse effects than mono exposure;
vi) pre-treatment with scavengers with capacity to capture ROS decrease the teratogenicity by different Ikr blockers (including PHT and TMD).

The drug-induced embryonic rhythm disturbances could theoretically explain the wide variety of birth defects observed after in utero exposure to PHT, PB, TMD and CBZ as follows:

1) Long-lasting bradycardia resulting in prolonged embryonic hypoxia. This may explain reported embryonic death, growth retardation, and mild CNS dysfunction and some minor structural abnormalities, related to decreased growth of skull bones.
2) Episodes of embryonic arrhythmia resulting in episodes of hypoxia/reoxygenation and ROS generation. This may explain severe birth defects, like orofacial clefts and limb reductions of varied severity. Distal phalangeal reductions seem like the easiest to induce after only a short episode of arrhythmia. All these defects are histologically preceded by oedema, vascular disruption, haemorrhage and necrosis.
3) Arrhythmia resulting in alterations in embryonic blood flow and blood pressure which may cause cardiovascular defects, including transpositions and absence of vessels.

In conclusion, a common pharmacological property (Ikr inhibition in the embryonic heart), may explain why PHT, PB, TMD and CBZ cause a very similar pattern of birth defects, despite these AEDs have different chemical configuration and metabolism.

2.3.4. Discussion[8]

Dr Tomson pointed out that the hypothesis, that drug induced embryonic arrhythmia leading to hypoxia-related damage is a common teratogenic mechanism for many AEDs, is based on a series of experimental and clinical observations. Some established teratogenic AEDs (TMD, PB, PHT and CBZ) have been associated clinically and experimentally with a similar pattern of adverse foetal effects including growth retardation, distal digital reductions, oro-facial clefts and cardiac defects:

1) The same pattern of developmental toxicity can be induced experimentally by hypoxia.
2) AEDs with hypoxia-like adverse foetal effects induce cardiac arrhythmia in a dose-dependent manner in cultured rat embryos and may thus cause episodes of interrupted oxygen supply to the embryo.
3) Sensitive periods are the same for AED-induced teratogenic effects and embryonic arrhythmia, and hypoxia induced adverse foetal effects.
4) Teratogenic doses of PHT induce embryonic cardiac arrhythmia in rats in vivo.
5) Finally, inhibition of the rapid component of the delayed rectifying K ion current (Ikr) is suggested as the fundamental pharmacological mechanism for the embryonic arrhythmia and the subsequent foetal adverse effects. The fact that other types of drugs that act on Ikr are associated with similar teratogenic effects experimentally is used as support for this hypothesis.

[8] Dr Torbjörn Tomson, Department of Clinical Neuroscience, Division of Neurology, Karolinska Institute, Stockholm, Sweden.

Although this series of observations is convincing there are some issues for discussion related to the clinical observations, experimental conditions as well as the interpretations. First, although the pattern of developmental toxicity varies between on the one hand valproate, which does not inhibit Ikr nor cause embryonic arrhythmia, and PB, PHT and CBZ on the other, there is also a considerable overlap in clinical studies. As examples, valproate has been associated with skeletal abnormalities and cardiovascular malformations, while CBZ probably does increase the risk of NTDs. Hence, other mechanisms are likely to contribute at least to some components of the spectrum of adverse foetal effects. Second, have the different experimental studies been performed at clinically relevant drug concentrations? The in vivo data on AED induced embryonic arrhythmia suggest so, whereas this is more difficult to assess in the in vitro experiments with cultured rat embryos. The concentrations at which dimethadione, PHT and PB were shown to inhibit Ikr are considered clinically relevant in relation to increased risks of cardiac arrhythmia such as torsade de pointes in the adult patient (Webster et al., 2002). How this relates to the embryonic heart is not completely clear, but the hypothesis rests on that the embryonic heart is more sensitive than the adult heart, and arguments supporting this assumption are presented. A third issue for discussion is how this proposed mechanism may relate to an individual genetic predisposition to teratogenic effects. Finally, it is important to consider if and how drug induced embryonic arrhythmia and hypoxia-related damage could be studied in the clinical setting.

In the general discussion Professor Lindhout commented that, Dr Danielsson had shown, many AEDs act on the Ikr channels, as these drugs are designed to act on electrophysiological properties of the central nervous system, so there may be something in common between these two mechanisms. This could imply that a genetic predisposition to epilepsy might also confer a genetic predisposition to teratogenesis. He also suggested that the AEDs may be teratogenic because they produce both ROS as well as making the situation worse by interfering with the foetal circulation.

Professor Chadwick commented that he was less concerned about the effects of PHT, and more interested in whether valproate has been shown to have the same effects in either a adult or human heart. Dr Danielson replied that the mechanism of valproate seems to be different as it does not appear to block Ikr or cause arrhythmia.

2.3.5. Folate

2.3.5.1. Drug interference with folate metabolism[9]. The early focus on the relationship between folic acid and AED therapy followed from the known pharmacological interactions that occurred between most of the frontline AEDs including: PB, PHT, primidone, and CBZ, which were known folate antagonists, and reduced the concentrations of this critical B-vitamin in the circulating plasma. Basically, any of the drugs that induced the cytochrome p450 enzyme system, were known to lower plasma folate levels.

The standard of care for pregnant women in the US is to receive a multi-vitamin containing folic acid (400 μg) daily in the periconceptional period to minimize the risk of congenital malformations. Folates are essential co-factors involved in one-carbon metabolism, participating in the biosynthesis of nucleic acids and the re-methylation of homocysteine to methionine, a key step for biomethylation reactions. In recent years, several epidemiological and intervention studies have demonstrated that periconceptional folic acid supplementation reduces the occurrence of various forms of congenital malformations, especially NTDs (Berry et al., 1999). With folates having key roles in several biochemical pathways and

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being metabolized by numerous enzymes, there are many circumstances during organogenesis that could be compromised by a folate deficiency, resulting in aberrant development and birth of a malformed infant.

The critical issue concerns the potential benefit of periconceptional supplementation with folic acid in preventing AED-induced birth defects. Hernandez-Diaz et al. (2001) studied 1242 case infants and foetuses with non-syndromic NTDs, using as controls 6660 malformed children with non-folate related structural defects as a reference group. She determined that the odds ratios for having an infant with an NTD following in utero exposure to an AED that was a folic acid antagonist was 2.8, with CI of 1.7–4.6. The adjusted odds ratios for in utero exposure to CBZ was almost three times that, OR = 6.9, with wide confidence intervals (Hernandez-Diaz et al., 2001). A follow-up study demonstrated that compounds that are folic acid antagonists and inhibit the enzyme dihydrofolate reductase increased the risk for an infant having a cardiovascular defect (OR = 3.4, CI 1.8, 6.4) or an oral cleft (OR = 2.6, CI 1.1, 6.1). Although these drugs were not the frontline AEDs, it at least demonstrated that the relative risk for cardiovascular defects associated with the use of the dihydrofolate reductase inhibitors in the absence of folic acid had a relative risk of 7.7 (CI 2.8, 21.7). These risks could be reduced to 1.5 (0.6, 3.8) with folic acid supplementation.

The issue then, is whether or not there is sufficient evidence in the literature at this point in time to suggest that folic acid can provide protection to the developing embryo against selected AED-induced birth defects. At the present time, there is no direct positive evidence that folic acid supplementation can provide protection against either valproic acid or CBZ-induced NTDs. Nonetheless, Professor Shorvon of the National Hospital for Neurology and Neurosurgery in London recently published (Shorvon, 2002) that it 'seems sensible to ensure folate is taken by pregnant women with epilepsy, although it has to be admitted that few studies have been carried out to show beneficial effects in epilepsy. Folate should be given at higher doses (4 mg/day is often recommended) than in non-medicated women owing to the diminished absorption of folate in-patients on hepatic enzyme inducing AEDs'. While this recommendation is not supported by 'evidence based medicine', it underscores the importance of continuing to recommend some level of folic acid supplementation to all women of reproductive age in order to provide protection from birth defects that are not secondary to the AED exposure.

2.3.6. *Discussion*[10]

Dr Dean said that evidence from the non-epileptic population suggests that the incidence or recurrence of certain malformations, whose inheritance is multifactorial, can be reduced by dietary folate supplementation. These same malformations are more common following anticonvulsant drug exposure in pregnancy. As these drugs interfere with folic acid metabolism, the folic acid pathway may be the common factor, although whether this operates by non-specific or specific effects on gene expression, through a toxic metabolite, or through metabolite deficiency remains to be determined. Any of these are likely to have underlying genetic determinants, so that investigation of these mechanisms may allow improved targeting of therapy.

In the general discussion the issue of what dosage of folic acid should be recommended was raised. Dr Hiilesmaa asked about potential toxic doses of folic acid and Professor Finnell replied that in a recent study doses up to 15 mg/day were quoted as being safe. Dr Morrow commented that although we do not know at present if folic acid is actually protective in this group, it is current wisdom that we should prescribe folic acid. However, data collected through the UK Epilepsy and Pregnancy register has shown that 1/3 of these women receive folic acid supplements preconceptionally. Professor Lindhout added that we should be careful about our prescribing practices due to our lack of knowledge, and warned off prescribing pharmacological doses of these supplements. One of the problems related to folic supplementation is

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Table 1
MTHFR and malformations

Malformation	Subject	667 C > T genotype	OR	95% CI
NTD	Infant	TT homozygote	1.8	1.4–2.2
	Mother	TT homozygote	2.0	1.5–2.8
Cleft palate	Patient	TT homozygote	3.23	1.32–7.86
CHD	Patient	CT heterozygote or TT homozygote	3.6	1.3–9.8

NTD, neural tube defect; CHD, congenital heart disease.

that a large number of pregnancies are unplanned, so the question arises as to whether all potentially 'at risk' women should be prescribed folic supplements during their child bearing years.

2.3.6.1. MTHFR mutations[11]. Various studies in the 1990s suggested that periconceptual folic acid supplementation reduces the frequency of certain birth defects, including NTDs, congenital heart disease, orofacial clefts and urinary tract (Wald, 1991; Czeizel, 1996). Recurrence risk is higher than incidence for these malformations, and the effectiveness of folic acid prevention varies between populations. This suggests a genetic susceptibility to folic acid sensitive malformations, and a natural candidate gene for this is 5,10 methylene tetrahydrofolate reductase (MTHFR; Ueland et al., 2001). Dietary folic acid is metabolized to 5-10 methylene tetrahydrofolate, a methyl donor for the pathways leading to DNA and RNA synthesis. MTHFR converts 5-10 methylene tetrahydrofolate to 5 methyl tetrahydrofolate, a cofactor in the DNA methylation pathway. DNA methylation is part of the mechanism of silencing gene transcription, and therefore, mutations in MTHFR may alter patterns of gene expression in the developing embryo, particularly in the presence of dietary folate deficiency, causing malformation. This could also provide a teratogenic mechanism for folic acid antagonist drugs, including AEDs.

2.3.6.2. MTHFR and malformations. The most widely investigated polymorphism in MTHFR is the 677C > T mutation, which results in an alanine to valine substitution and a 'thermolabile' enzyme with reduced activity at 37 °C. Another is 1298A > C which has a lesser effect on enzyme activity. In most Northern European populations, 677C > T is common, around 12% (8–18%) of people being homozygous (Botto and Yang, 2000). Such individuals have mildly raised blood homocysteine if their folic acid intake is poor. A number of association studies investigating MTHFR genotypes and malformations have been undertaken.

A meta-analysis of studies of the 677C > T MTHFR mutation and NTDs (Botto and Yang, 2000) suggested an association with both the affected infant and the mother's genotype (Table 1). The evidence that the 1298A > C allele confers risk for neural tube defect is inconclusive. A Californian study of MTHFR and orofacial clefting found no association, but a later Irish study found an association between isolated CL(P) and homozygosity for 677C > T (Table 1), but no association for cleft lip and palate. A small prenatal study of congenital heart disease demonstrated higher amniotic fluid homocysteine in affected pregnancies, and an association with foetal homozygosity or heterozygosity for 677C > T (Table 1). No MTHFR association studies have yet been published for urinary tract malformation, nor for limb reduction defects. A recent study of mothers who took folic acid antagonists in pregnancy (Hernandez-Diaz et al., 2000) including trimethoprim and some AEDs found a relative risk of urinary tract defects of 2.5 (95%CI 1.2–5). Similarly, there is some evidence that limb reduction defects may be preventable by multivitamin supplementation including folic acid, and an hereditary brachydactyly in rabbits is preventable by folic acid and vitamin B12 supplementation.

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2.3.6.3. MTHFR and AED therapy. The major malformations affected by maternal folic acid status such as NTDs, orofacial clefting and congenital heart disease are also those attributed to the teratogenic effects of AEDs. Epileptic mothers taking AEDs such as PHT, PB and CBZ have lower blood levels of folic acid than controls, and mild hyperhomocysteinemia occurs in some cases. Valproic acid is also known to alter folic acid metabolism. Taken together, these findings suggest that part of the increased risk of malformation in this situation could be attributable to folic acid deficiency, perhaps interacting with the maternal genotype, including MTHFR. FAS has been associated with the maternal MTHFR genotype (OR 3.2, 95%CI 1.02–10.04), with some additional association between FAS related childhood medical problems and the child's genotype ($P < 0.05$, χ^2-test; Dean et al., 1999). Most of these children had foetal valproate syndrome. FAS may, therefore, arise from a complex interaction involving both the child's and the mother's genotypes, in addition to other factors such as maternal nutritional status, AED used and the dosage.

2.3.6.4. MTHFR and Down's syndrome. A number of studies have investigated a possible link between folic acid pathway genes and the risk of Down's syndrome. Chromosomal aneuploidy is characteristic of many malignant tumors, as is DNA hypomethylation. Aberrant chromosome segregation is a cardinal feature of the rare autosomal recessive disorder, Immunodeficiency, Centromere Instability and Facial anomalies (OMIM 242860), caused by mutation in the DNA methyltransferase 3B gene. In one study, Down's syndrome was associated with maternal MTHFR mutation, but in another, no association was found, except when the methionine synthase reductase genotype was also taken into account. Another study demonstrated an association with trisomy 18, but not with sex chromosome aneuploidy, nor with non-viable autosomal trisomies. The link with meiotic non-disjunction remains uncertain.

2.3.6.5. Mechanisms of teratogenesis. MTHFR might influence embryonic development in at least two ways. Firstly, the rise in plasma homocysteine associated with MTHFR mutation or folic acid deficiency could be embryotoxic, although the mean rise is only 2.6 μm/l in a 677C > T homozygote. Raised amniotic fluid homocysteine occurs in some neural tube defect pregnancies with this genotype. Homocysteine has also been shown to inhibit the *N*-methyl-D-aspartate (NMDA) glutamate receptor. This receptor is widely expressed in neural crest cells, and other NMDA antagonists are known to be teratogenic. Alternatively, an effect on DNA methylation could affect control of gene transcription. Repression of gene expression occurs when a regulatory element such as MECP2 binds to methylated DNA, and recruits co-repressors including histone deacetylase. Insufficient substrate for DNA methylation could thus cause inappropriate gene expression in the embryo with teratogenic results. This mechanism would also be sensitive to folic acid intake, and could explain the protective effects of folic acid supplementation, and the detrimental effects of folic acid antagonists. The folic acid micronutrient hypothesis (some cells may have higher metabolic requirements for folic acid than others), may help explain some of the malformation specificity of folate deficiency and MTHFR. Other teratogenic mechanisms are likely operate simultaneously for AEDs, including liver enzyme induction, the effects of toxic metabolites, and direct effects on other genes. Valproic acid, for example inhibits histone de-acetylase directly, providing another route of interaction with the folic acid pathway and MTHFR. It is likely that AED teratogenesis is a complex process, with more than one underlying risk factor.

2.3.7. Discussion[12]

Professor Finnell said that, given the epidemiological association linking maternal periconceptional multi-vitamin consumption and a reduction in the risk for NTDs, a number of specific folate pathway genes have been targets of serious in-

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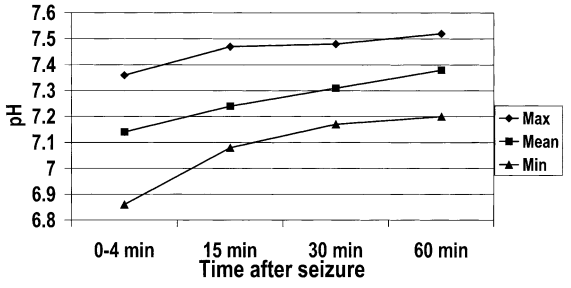

Fig. 2.

vestigation. None has received quite the attention as MTHFR, which is highly polymorphic, such that certain genotypes have elevated homocysteine and reduced plasma folate concentrations. Given that many frontline AEDs are folate antagonists and can lower folate concentrations as well as induce NTDs, Dr Dean theorized that individuals with the MTHFR 677TT haplotype would be at increased risk for an adverse pregnancy outcome following in utero exposure to AEDs. Based upon his 1999 publication, he observed a 3-fold elevated risk associated with MTHFR 677T homozygous epileptic mothers having affected children. There are design issues pertaining to the selection of the case population and the control population that limit the ability to draw causal inferences from these data. Moreover, the study as designed, prevented the investigators from being able to disentangle whether a woman's MTHFR genotype was predictive of foetal outcome, or was associated with her epilepsy. Nevertheless, these data contribute to the emerging body of evidence that susceptibility to AED teratogenesis is highly complex and involves multiple genetic pathways and environmental interactions.

In the general discussion Professor Wells asked about the pattern of birth defects caused by homocysteine and whether any behavioral phenotypes are seen. Professor Finnell replied that although homocysteine caused phenotypes in chick experiments he had not personally seen any in mammalian models. In his folate knock out mice the homocysteine levels get very high without any obvious adverse effects, however, he had not looked at behavioral problems. Dr Dean remarked that the rise in homocysteine levels seen in humans with the MTHFR mutations was actually very small. Dr Clayton-Smith mentioned that there was a human MTHFR deficiency which causes neurodevelopmental phenotypes which are quite Angelman like, but the affected children do not show structural abnormalities.

2.3.8. *Maternal seizures*[13]

Profound alterations in acid-base equilibrium occur during and immediately after a grand mal seizure. The mean arterial pH drops promptly, and can in some patients be as low as 6.9 (Orringer et al., 1977). This acidosis is largely attributable to an elevated serum lactate concentration, which rises to about 10-fold (Orringer et al., 1977). Lactate is released from muscles during and after their intensive activity during convulsions.

Fig. 2 shows the time course of arterial pH during the postictal period in eight patients (Orringer et al., 1977). The resolution of low pH occurs within an hour, whereas it takes several hours until the lactate level decreases to normal

These changes in maternal acid–base equilibrium are most probably rapidly mediated through the placenta to the foetus. There is no direct evidence on this in the form of foetal blood gas analyses, but foetal heart rate alterations registered in labor during a maternal grand mal seizure suggest acidosis (Teramo et al., 1979). It is, therefore, conceivable that the foetus becomes acidotic also during maternal seizures in pregnancy.

Foetal hypoxia and very low pH at birth are known to be associated with disturbances in development. Animal studies show that heavy lactic acidosis can be harmful to many organs. Therefore, there is a good reason to avoid seizures in human pregnancies.

2.3.8.1. Circulatory and mechanical effects of seizures. During a tonic–clonic convulsion, blood pressure is elevated and a redistribution of circula-

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tion takes place with blood flow to the brain and muscles increasing, and there is evidence that blood flow to visceral organs (e.g. kidneys) is decreased. It is, therefore, conceivable that blood flow to the uterus is also reduced, although direct evidence for this is lacking. The increased intra-abdominal pressure during a seizure may further reduce uterine circulation.

If the patient falls during a grand mal seizure there is a risk of uterine contusion and subsequent placental abruption. Although early reports suggested that there is a slightly elevated risk of miscarriages and many obstetric complications in women with epilepsy, these results could not be verified in most of the recent clinical studies (Hiilesmaa, 1992).

Virtually all published reports agree that there is a 1.2–3-fold increase in the rate of perinatal mortality (stillbirths + deaths during the first week of life) among offspring of WWE (Hiilesmaa, 1992). However, no direct relationship between maternal seizures and perinatal deaths has been observed and the increased rate of perinatal deaths in the offspring of WWE is probably multifactorial (Hiilesmaa, 1992).

It is obvious that partial seizures and non-convulsive generalized seizures are not followed by the metabolic and circulatory changes seen after a grand mal seizure. It is, therefore, most unlikely that these seizure types expose the foetus to immediate risks in utero.

2.3.8.2. Foetal effects of seizures. The possible teratogenic effects of seizures during pregnancy have been addressed in several clinical studies and to date no such effect has been demonstrated. The existing data suggests that the increased rate of teratogenesis in offspring of WWE is due to ADEs rather than to seizures in pregnancy (Holmes et al., 2001).

Despite the general efficacy of the modern AEDs, seizures during pregnancy cannot be completely avoided. We observed in Helsinki that in 22% of 784 pregnancies in women with grand mal epilepsy, at least one generalized tonic–clonic convulsive seizure occurred during pregnancy. These seizures were not followed by any immediate foetal complication, and it thus seems that a healthy foetus is remarkably resistant to the transient albeit profound metabolic acidosis. A hazardous situation can, however, develop if a seizure occurs in a pregnancy already complicated by a pre-existing obstetric problem, such as placental insufficiency or chronic foetal hypoxia.

The risk of psychomotor retardation and the prevalence of mental subnormality are somewhat increased in offspring of mothers with epilepsy (Granström and Gaily, 1992). This probably results from many prenatal and postnatal factors connected with maternal epilepsy including genetic predisposition, antiepileptic medication and socio–economic elements. In the light of current knowledge, it seems rather unlikely that maternal seizures during pregnancy have important detrimental long-term effects on foetal development (Granström and Gaily, 1992). Due to the complexity of carrying out developmental studies, the currently available series is still rather small. Some more results are needed to exclude, with a reasonable confidence, effects of maternal seizures on the child's development.

2.3.8.3. Seizures in labor. In 1–2% of women with grand mal epilepsy, a seizure occurs during labor (Hiilesmaa, 1992). The probability of a seizure during labor is nine times greater than that during pregnancy. Stress, pain during uterine contractions, lack of sleep, and poor drug compliance are all factors that can lower the seizure threshold during labor.

Cases in which foetal cardiotocography during labor has been carried out during a maternal seizure have elucidated the events that occur in the foetus. A maternal tonic–clonic seizure is immediately followed by foetal bradycardia and reduced short-term variation, which last about 15 min. Later on, tachycardia and late decelerations can follow (Teramo et al., 1979). These changes in foetal heart rate are suggestive of foetal acidosis and hypoxia. Similar changes are also seen after an eclamptic seizure and in other obstetric complications causing foetal hypoxia and acidosis.

The increased foetal risks and the postictal inability of the mother to further cooperate in vaginal labor are good reasons for a caesarean section if a seizure has occurred during labor, but

each case should be individually evaluated. Due to the risk of seizures during labor, delivery of mothers with epilepsy should take place at large enough obstetric units with sufficient facilities.

During the general discussion, Professor Richens enquired if there were any studies that had looked particularly at the teratogenic effects when seizures had taken place during the time of organogenesis. Dr Hiilesmaa replied that there were three or four clinical studies that had looked at this specific question and had found no adverse effects, however, the lack of power in these studies makes it difficult to draw a conclusion. Professor Meador raised the issue of a need for women to be presented with a balanced view taking into account the risk of the seizures to themselves as well as the risks of the AEDs to the foetus. Professor Lindhout suggested that a possible reason for no evidence of a teratogenic risk of maternal seizures could be that there is such a large range of phenotypes that it is difficult to collate. However, other experimental models on these types of metabolic changes have shown detrimental effects on the foetus, so lack of evidence should not necessarily be a reason to state that there is no risk to the foetus.

Professor Chadwick presented some important data looking at the effects of seizures during pregnancy on the mother. The UK regularly conducts confidential enquiries into maternal deaths. Looking at data from five reports covering 1985–1999, there were around 1000 deaths from 11 million pregnancies. Around 50 of these deaths were to women with epilepsy, making epilepsy the third most common cause of indirect death, with only cardiac deaths and stroke being more common. This equates to a ten times increase in mortality over this 15 year period for women with epilepsy. Although a slight increase would be expected in view of the fact that the standard mortality rate for epilepsy across the whole age spectrum is 2–3, it is obvious that something is happening during pregnancy in epileptic women to increase the mortality rate.

In fact, most of these deaths are seizure related and the most common scenario is a woman who discovers she is pregnant and at that point decides to discontinue her medication. This represents the worst of both worlds, because by the time the woman knows that she is pregnant most of the embryonic organogenesis will have already taken place, so stopping her drug treatment abruptly will not reduce the risk of congenital malformations but will put the mother's life at risk from seizures provoked by drug withdrawal.

If we assume that around 5% of pregnancies in WWE are associated with a malformation, there are around 5000:100 000 of these to set against 100:100 000 maternal deaths. The difficult question is how many malformations justify a maternal death.

2.3.9. *Maternal genes*[14]

Little attention has been devoted to the fact that congenital malformations in children born to mothers with epilepsy could have other aetiologies than direct fetotoxicity of AEDs to which they were exposed in utero. A few studies have focused on the occurrence of malformations (cleft lip with/without CL(P) or congenital heart defects) in near relatives of children exposed to AEDs during intra-uterine life.

Müller-Küppers (1963) reported on a cleft lip and palate in a child exposed to AEDs in utero. A year later Janz and Fuchs (1964) published a brief paper on 358 children of mothers with epilepsy; 225 of the children had been exposed to AEDs during the first trimester of intra uterine life, due to the treatment of the epilepsy of their mothers.

Only five malformed children were found and they concluded that AEDs were not a serious risk factor in the aetiology of congenital malformations in children exposed to AEDs in utero. However, a detailed analysis of the individual malformations reported suggests that the risk of cleft lip with or without CL(P) and cleft plate(CP) was increased approximately 5–6 fold compared with background population values. No family histories on the occurrence of facial clefts were reported.

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Meadow (1968) reported in the Lancet that many children born with facial clefts had mothers with epilepsy. This finding was subsequently confirmed by many authors (Dronamraju, 1970; Pashayan et al., 1971; Erickson and Oakley, 1974).

Thus, in the aftermath of the thalidomide scandal, the main issue of the early studies of congenital malformations, epilepsy and AEDs, was teratogenicity.

More than 20 years after the first reports on AED-teratogenicity, the possible genetic association between epilepsy per se and the individual malformations (CL(P)/CP) was explored (Kelly 1984; Friis et al., 1986).

2.3.9.1. Facial clefts. Facial clefts comprise CL(P) (cleft lip with or without CL(P)), CP CL(P) and the rare median clefts.

CL(P)/CP are among the most frequent serious congenital malformations with an incidence of approximately two per 1000 newborns in the general population.

Several studies have suggested that AED-treated maternal epilepsy is a risk factor for the development of CL(P)/CP in the children. However, twin and family studies have also shown, that there is a strong genetic component in the aetiology of these malformations (Fogh-Andersen, 1942). Therefore, at least not all facial clefts in children of AED-treated epileptic mothers can be attributed to a teratogenic effect of the AEDs. The genetic family background have to be scrutinized for other cases of CL(P)/CP.

Annegers et al. (1974), Shapiro et al. (1976) found indications that paternal epilepsy also increased the risk of congenital malformations in the offspring, whereas no evidence of an increased CL(P)/CP-risk was found.

In 1979, Friis (1979) reported on maternal and paternal epilepsy and first trimester AED-exposure in 391 live-born children with CL(P) or CP. Eighteen persons with epilepsy (seven fathers and 11 mothers) were identified. The point prevalence of epilepsy among parents of CL(P)/CP patients is thus 2.3%, or approximately three times expected values. No statistical difference between the numbers of fathers and mothers with epilepsy was found. The pattern of the AED-treatment was no different from expectation. Thus this result suggested that epilepsy per se could play a role in the genesis of CL(P)/CP in the offspring of patients (males and females) with epilepsy.

Kelly et al. (1984) found 13 parents with epilepsy (11 mothers and two fathers) among 175 probands with isolated CL(P) and no cases of parental epilepsy among 140 probands with other clefting than CL(P), including CP.

Greenberg et al. (1977) in a case-control study on maternal drug histories found a significantly increased exposure rate of PB among 412 malformed infants with CL(P)/CP. The statistical significance of the PB correlation, however, was lost when mothers with a close family history of the birth defects in question were excluded.

Friis et al. (1981) identified 11 patients with facial clefts among 3203 children of epileptic mothers, whereas only five were expected using Danish population-based incidence figures. This suggests an association between facial clefts and epilepsy per se. Thus when a child with CL(P)/CP exposed to AEDs during the first trimester of pregnancy, is born, a thorough family history on CL(P)/CP should be taken (Friis et al., 1981, 1986).

2.3.9.2. Congenital heart defects. CHDs are amongst the malformations most frequently associated with first trimester AED-exposure.

Anderson (1976) was one of the first to report an increased incidence of CHD in offspring of AED-treated mothers with epilepsy. He found 18 cases of maternal epilepsy amongst approximately 3000 mothers of children with CHD. All these children were seen at the University of Minnesota Hospital from 1961–1975. He concluded that the rate of CHD in children prenatally exposed to AEDs were increased. However, if those two children exposed to the known teratogens (which is TMD and paramethadione) and those two patients initiating the study are excluded (in all six patients), the CHD-incidence is approximately 1:200 or close to expectation in most countries with population-based birth defect registries. Anderson (1976) found a wide range of CHDs among the 18 children of mothers with epilepsy, but noted an apparent skewing of the distribution towards

VSD and pulmonary stenosis in combination with other defects, suggesting a malformation specificity involving these two CHDs. No AED specificity was reported.

Friis and Hauge (1985) in a large group of children of parents (mothers and fathers) found no evidence of an increase in the incidence of CHDs.

It should be noted, that the genetics of CHDs is even more complex than that of CL(P)/CP. Future studies on CHD genetics may help to solve some of the problems associated with prenatal AED-exposure, including counseling of parents with epilepsy.

2.3.9.3. Neural tube defects. The aetiology of NTDs are influenced by both genetic and environmental factors.

Robert and Guibaud (1982) in their first publication linking valproate (VPA) exposure to NTD, noted that three of nine infants with NTDs had a close relative with spina bifida.

Rosa (1991), Lindhout et al. (1992a) presented evidence that CBZ- exposure in the first trimester increased the risk of having children with NTD, however, not as frequently as with VPA (Omtzigt et al., 1992). Lindhout et al. (1992b) analyzed the spectrum of NTDs in 34 infants prenatally exposed to AEDs (preferably VPA and CBZ). They found an anencephaly to lumbosacral spina bifida aperta ratio of 1:33, suggesting a specific association with caudal CNS defects (=malformation specificity). Other midline defects were also associated with VPA (hypospadias, hypertelorism, partial agenesis of the corpus callosum and agenesis of the septum pellucidum with lissencephaly). No specific association with either maternal family history of NTDs or epilepsy was found.

Klepel and Freitag (1992) examined 182 children and adolescents with epilepsy (age: 3–18 years) for the presence of closed spina bifida. They found, in contrast to the background population, that sacral localization was predominant among epilepsy patients and that, patients with idiopathic epilepsies had the highest frequency of spina bifida, suggesting an association between closed sacral spina bifida and idiopathic epilepsy.

The issue on the MTHFR gene in fetal abnormalities, especially NTDs has been covered earlier.

2.3.10. Discussion[15]

Professor Lindhout reminded the participants that four of the major principles of teratology are (adapted from Wilson, 1977)

1) The susceptibility to teratogenesis depends on the genotype of the conceptus and in the manner in which this interacts with environmental factors.
2) The susceptibility to teratogenic agents varies with the developmental stage at the time of exposure.
3) The access of a teratogen to developing tissues (pharmacokinetics) and the way it acts with its embryonic or foetal target (pharmacodynamics) depends on the structure of the agent (structure–activity relation).
4) The manifestations of deviant development depend on the dose of the agent (dose–effect relation).

Arguments from all four principles point towards AED induced teratogenesis in man as a very likely occurrence. The increased risk of adverse pregnancy outcome in maternal epilepsy is usually attributed to maternal AEDs use, but one has to take into account the potential role of genetic predisposing factors. These may consist of maternal genes involved in the etiology of epilepsy, and other abnormalities such as pleiotropy and variable gene expression.

However, paternal genes that segregate independently from 'epilepsy genes', and genes conferring pharmacokinetic or pharmacodynamic diversity between exposed individuals may also play a role. Those who repudiate AED induced teratogenesis, usually base their assumptions on the suggestion that maternal epilepsy is caused by factors that also cause abnormalities in the off-

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spring and quote observations of a more frequent family history of malformations in affected offspring as compared with unaffected offspring. However, they disregard that most if not all data on family histories are biased by the outcome and that all others arguments strongly suggest that most if not all AEDs are associated with teratogenesis.

The studies by Friis et al. have the advantage that they are population-based through registries for which the Nordic countries are well-known. However, these registries are retrospective which does not solve the problem of a family history that is biased by knowledge of the pregnancy outcome. This type of bias can be partly controlled for by comparing the family histories of the female epilepsy patients with those of their male spouses. In one such study of NTDs after maternal AED use, the paternal relatives and maternal relatives turned out to be equally affected with respect to NTDs or primary generalized epilepsy (the epilepsy type considered to be determined proportionately more by genetic factors; Lindhout et al., 1992a,b). From that study the conclusion was drawn that genetic predisposing factors for AED induced teratogenesis may play a role, but probably independent from maternal epilepsy genes.

Other potentially strong confounders are disease related selective partner choice, differential reproductive fitness, and—when observing postnatal development—medication effects on maternal educative capabilities. Studies that compare offspring of mothers with epilepsy with offspring of fathers with epilepsy have been put forward as a mean to control for the contribution of epilepsy genes. However, men and WWE may differ significantly with respect to partner choice and reproductive fitness. This implies that those men and women who indeed do reproduce are difficult to compare unless one is able to sufficiently stratify for epilepsy aetiology (which until to date remains unknown in the vast majority of women with epilepsy).

During the general discussion Professor Friis agreed with Professor Lindhout that selective partner choice was indeed very important and could have a large influence on malformation rates in families like this. Dr Clayton-Smith commented that although we know of many genetic syndromes which result in CL(P), we should be careful about over estimating these as there are not many which can be transmitted in a dominant manner. The ones that have been seen in relation to epilepsy have tended to be de novo mutations so would not have any implication when thinking about maternal genes and risk of teratogenicity. She further commented that due to the advances that have been made in genetic analysis over recent years it is worthwhile sending these families for genetic analysis. Professor Finnell added that as around 1:5 genes are involved in making the face and the number of genes known to be associated with epilepsy is approaching 100, the overlap of these groups is going to be very large, so the advent of proteomics and genomics is going to be very important in clinical practice.

Professor Lindhout raised concerns about the way that the data in these types of studies was presented and analyzed. He felt that it is important that full genetic screenings are carried out on these families.

3. Psychomotor development

Early case reports of children exposed to AEDs in utero suggested a relatively high incidence of mental retardation. Both growth retardation and multiple minor anomalies have been reported in infants exposed to AEDs and there seems to be an association with impaired intellectual function. The results of these studies have not been consistent and this may be due to the widely varying populations studied and methods used. Minor anomalies, for example, become less obvious in children followed up for a long period of time and problems with psychomotor development may become more or less obvious. Recent prospective studies have been more rigorously designed and these have suggested that the incidence of mental retardation may not be a high as originally feared. Rather than a global retardation there may be specific deficits, which only become apparent at school age.

3.1. Drugs effects

3.1.1. Published studies of psychomotor development[16]

The brain is vulnerable to teratogenic agents such as AEDs throughout pregnancy. Exposure in the first trimester may cause major malformations, whereas in the second half of pregnancy, teratogenic effects may disturb neuronal migration and synaptic organization, and result in mild to moderate cognitive dysfunction. Long periods of follow-up and the relatively laborious methods of measuring cognitive abilities increase confounding factors. Consequently, most published reports on development of children born to mothers with epilepsy include a limited number of subjects and the evaluation of drug effects has been difficult.

Other factors besides drug exposure that may affect development of children of mothers with epilepsy include maternal seizures during pregnancy, genetic traits associated with epilepsy and psychosocial problems associated with frequent seizures. Prolonged maternal generalized tonic–clonic seizures may cause foetal brain damage, especially in the later stages of pregnancy. There is no evidence, however, that brief convulsive seizures or nonconvulsive seizures would cause cognitive problems in the exposed children. Some genetic traits such as tuberous sclerosis are known to cause both epilepsy and developmental problems, but these are very rare in child bearing women.

Four prospective population-based studies have investigated intelligence or psychomotor development at the age 4–7 of years. Three studies (Shapiro et al., 1976; Hanson et al., 1976; Gaily et al., 1988) reported intelligence scores obtained by WPPSI or WISC, and one study (Wide et al., 2002) used the Griffiths' test. Most of the data deal with PHT and PB exposure (more than 300 children), but also include 70 children exposed to CBZ, 40 nonexposed children of mothers with epilepsy and over 28 000 control children (mainly from the Collaborative Perinatal Project). The mean intelligence scores in children of mothers with epilepsy were slightly lower than those of control children, but this could not be attributed to drug exposure. The only study reporting significantly lower intelligence in children exposed to PHT (mainly in polytherapy) (Hanson et al., 1976) did not have a control group of nonexposed children of mothers with epilepsy. Wide et al. observed an impairment in locomotor function in children exposed to PHT monotherapy but no significant differences in the other subsets of the Griffiths's test. One study (Gaily et al., 1988) estimated the risk of mental deficiency which was found to be slightly increased with multifactorial aetiology in children of mothers with epilepsy.

There are also four long term prospective clinic-based studies published between 1984 and 1999 that measured cognitive abilities in a total of 206 drug exposed, 17 unexposed and 221 control children. One study included monotherapy (PHT or primidone) and polytherapy exposures as well as nonexposed children of mothers with epilepsy and controls, and found that those exposed to polytherapy had significantly lower IQ-scores than the other groups. Another study calculated a drug score based on doses (mg) and number of AEDs used and they found that children with higher drug scores had poorer developmental quotients. Two studies focused on CBZ monotherapy and reported contradictory results. One study found significantly lower IQ in 41 CBZ exposed children compared with 47 matched controls, while another study found no difference between 36 children with CBZ exposure and 70 matched controls. In the latter study, children exposed to PHT monotherapy performed worse than controls and children with CBZ exposure. The preliminary results of our own recent study (report in preparation) showed no IQ decrement in 86 children exposed to CBZ monotherapy.

There are no prospective IQ data available for children exposed to valproate except for the very small group of eight children (who showed no IQ

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reduction) reported by Gaily et al. (1988). Obviously, as valproate is a fairly common AED and known to increase the risk of malformations in the central nervous system, large prospective studies are needed to evaluate the risk that maternal valproate treatment may cause for later cognitive development in the prenatally exposed offspring.

Specific cognitive deficits (not related to mental deficiency) in children of mothers with epilepsy have been investigated in only one prospective study using subtests from WPPSI, the Leiter scale, ITPA and a neuropsychological test battery (later published as NEPSY). An increased risk of specific cognitive dysfunction was found in 104 children of mothers with epilepsy compared with 105 controls. The increased risk was not associated with drug exposure which was mainly to PHT. Another prospective study reported no difference in non-optimal school career when 56 children of mothers with epilepsy (22 nonexposed) were compared with 54 matched controls. A recent large retrospective survey (Adab et al., 2001) found an excess of additional educational needs in children with prenatal exposure to valproate in mono-and in polytherapy, raising concern that valproate may be harmful for later cognitive development in prenatally exposed children.

In conclusion, population-based studies give no evidence that prenatal exposure to PHT or CBZ would impair intelligence. Clinic-based studies and a large retrospective survey suggest that maternal polytherapy and valproate treatment may be harmful for cognitive development in the offspring. Children of mothers with epilepsy have an increased risk of specific cognitive dysfunction; the cause for this is not clear.

3.1.2. *Liverpool study*[17]

A number of studies have documented the association between specific AEDs and cognitive development; valproate (VPA), and polytherapy have all been associated with psychomotor delay, an increase in special educational needs and low intellectual functioning (Adab et al., 2001; Hill et al., 1982; Koch et al., 1999; Rovet et al., 1995; Mawer et al., 2002). Other studies have documented an association between multiple minor anomalies in children exposed to AEDs and delayed cognitive functioning (Gaily et al., 1988). Furthermore, a recent study reported that children exposed, in utero, to over l000 mg of VPA monotherapy had multiple anomalies and developmental delay (Mawer et al., 2002).

The question of whether minor anomalies and DF are predictors of a child's future intellectual development remains uncertain. A retrospective study in Liverpool has investigated the long-term effects of intrauterine AED exposure on neurodevelopment and DF in school age children born to WWE.

Between January 2000 and May 2001, women with a diagnosis of epilepsy who had children aged between 6 and 16 years 11 months were recruited. Women with learning difficulties, progressive neurological deficit or symptomatic generalized epilepsy were excluded. One hundred and fifty six women and 249 children were assessed.

The following neuropsychological tests were administered to assess different facets of intelligence, behavior and everyday memory functioning for each mother–child pair: the Wechsler Intelligence Scale for children (WISC-IIIuk), the Rivermead Behavioral Memory Test (RMBT) and the Vineland Adaptive Behavior scale (VABS). The National Adult Reading Test (NART) was applied to each mother to provide a pre-morbid measure of intelligence. Each child had a medical examination and photographs were taken of their feet hands and face. The facial photographs were assessed by geneticists, who were blinded to the drug exposure, to provided an overall gestalt score ranging from 0 to 10 for dysmorphic facial features associated with the FAS. Further information was collected including: maternal epilepsy type, maternal drug therapy including any other medication taken i.e. folic acid, frequency of seizures and socio-economic information.

Two hundred and forty nine children were assessed, 136 (55%) males and 113 (45%) females with a mean age 10.4 years, 41 were exposed to VPA, 52 to CBZ, 21 to PHT, 49 to polytherapy

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Table 2
Mean intelligence quotient (IQ) and 95% CI for all drug groups

	N	Verbal IQ	Performance IQ	Full Scale IQ
Valproate	41	83.61, CI 78.20–89.02	93.95, CI 88.98–99.01	87.17, 81.86–92.48
Carbamazepine	52	94.08, CI 89.61–98.54	89.40, CI 84.78–94.03	91.10, CI 86.36–95.83
Phenytoin	21	98.48, CI 90.56–106.39	97.14, CI 91.69–102.60	97.62, CI 90.26–104.98
Polytherapy	49	89.16, CI 85.10–93.23	89.16, CI 85.10–93.23	87.88, CI 83.93–91.83
No AEDs	80	90.90, CI 87.24–94.56	90.20, CI 86.10–94.30	89.48, CI 85.54–93.41

and 80 were unexposed. As shown in Table 2, children exposed to VPA had a significantly lower mean verbal IQ (83.61, CI 78.20–89.02) than the non-exposed group (90.90, CI 87.24–94.56) and the CBZ group (CBZ, 94.08 CI 89.61–98.54) and had the lowest full scale IQ when compare with all other drug groups (Table 2). A multiple regression model identified the significant factors that accounted for 30% of the variance on VIQ, the mother's intelligence had a positive effect (β co-efficient.418), exposure to VPA monotherapy had a negative effect (β co-efficient −0.165) and more than five tonic–clonic seizures had a negative effect on VIQ (*P* co-efficient −0.168). The following negative correlations were found, valproate dose and VIQ $r = -0.394$ ($P = \leq 0.05$), DF and VIQ $r = -0.392$ ($P = \leq 0.05$). VPA dose and DF had a positive correlation $r = 0.519$ ($P = \leq 0.01$). A dose effect was found, using univariate regression, for VPA exposure in the 1st trimester with a β co-efficient 0.038 significant at $P = \leq 0.05$. Exposure to VPA was associated with an increase in memory impairment, with 52% categorized as having low memory functioning, 44% had received speech therapy and 41% had been registered as having Special Education Needs (SEN).

The authors recognize there are limitations and biases of this study that should be mentioned. It was a retrospective hospital based study and, therefore, may have been subjected to a self-selection bias, in that mothers who have suspected problems with their children may have been more willing to take part in the research. It is acknowledged that these factors may compromise the external validity of the results due to the degree of patient selection.

In conclusion, therefore, it appeared that children exposed to VPA showed the largest effect having the lowest FSIQ and VIQ compared with other groups. VPA drug dose was found to have a negative effect on VIQ and a positive effect of DF, this supports previous research that suggested a high number of minor anomalies were associated with a lower mean IQ (Gaily et al., 1988). These findings may suggest that DF could be early indicators of developmental delay in children exposed to AEDs. A recent study reported that exposure to >1000 mg had been associated with an increase in anomalies and developmental delay (Mawer et al., 2002), our results were consistent with these findings reporting that 24% of children, exposed to >1000 mg of VPA in the 1st trimester had moderate to severe DF and were classified as mentally impaired. No teratogenic effect of seizures during pregnancy has been demonstrated (Hiilesmaa, 1997), however, more than five tonic-clonic seizures during pregnancy was found to have a significant negative effect on cognition. Exposure to VPA monotherapy had a negative effect on memory functioning and was associated with an increase in behavioral problems, SEN and DF when compared with other AEDs. Further research is needed in order to adequately quantify the adverse cognitive effects of exposure to AEDs in utero and the authors are in the process of conducting a longitudinal prospective study of children born to WWE in an attempt to address this problem.

3.1.3. Long term consequences of prenatal exposure to phenobarbital and phenytoin[18]

Genital anomalies have been reported in drug-exposed neonates (Pinto et al., 1977; Lindhout et al., 1992a,b) as well as in rodents (Gupta et al., 1982). In adult rodents prenatal exposure to PB and PHT affected reproductive functioning (Gupta et al., 1982, 1980) and altered sex dimorphic behaviors (Reinisch and Sanders, 1982). In order to determine whether these influences also be observed in human adults, a retrospective study of head size and genital anomalies in children who had been prenatally exposed to the anticonvulsants PB and was undertaken in the Amsterdam, Netherlands. A follow-up of cognitive abilities, reproductive functioning, gender development in adulthood and sexual orientation (the last two being the human equivalents of the altered sex dimorphic behaviors found in rodents) was also undertaken to investigate whether the cognitive impairments found in preschool children (Dessens et al., 1994) persist into adulthood.

The retrospective study included 198 (95 males, 103 females) anticonvulsant-exposed and equal numbers of matched control subjects. In the follow-up 147 (72 males, 75 females) anticonvulsant and 147 matched control subjects participated. No differences between groups were found on the subjects socioeconomic status or on parental educational level or socioeconomic status. Prenatally PB+PHT exposed neonates had a significantly smaller occipitofrontal circumference (OFC) at birth. The OFC of the PB+PHT neonates was around the 10th percentile, the OFCs of the remaining groups were around the 50th percentile. Groups did not differ on the cognitive tests or educational level. However, 12% of the exposed versus 1% of the control subjects had persisting learning problems and had been admitted to special schools for children with such problems. Subjects with learning problems performed significantly lower on the cognitive tests (Dessens et al., 2000).

Of the PB-exposed boys 15% had undescended testes at birth versus 2.8% of the PB+PHT-exposed and control boys. More anticonvulsant-exposed (24%) than control males (11%) had received medical treatment for genital anomalies. There was no association between a particular anticonvulsant therapy and genital anomalies.

Anticonvulsant-exposed females more often had irregular menstrual cycles (31 vs. 17%) and bleeding (15 vs. 3%) and reported more complications requiring obstetric care during pregnancy (Dessens et al., 2001).

Exposed and control subjects did not differ with respect to gender role behavior although higher numbers of prenatally anticonvulsant-exposed subjects reported current or past cross gender behavior and/or gender dysphoria. Three prenatally anticonvulsant-exposed subjects were transsexuals and had undergone sex reassignment surgery, a remarkably high rate given the rarity of transsexualism. There was one male to female transsexual and two female to male transsexuals. Neither group differed on sexual orientation; the majority reported heterosexual feelings and behavior, except for two exposed males who had exclusively homosexual experiences (Dessens et al., 1999).

From these findings we concluded that:

1) The combination of PB+PHT affects the foetal OFC.
2) Most of the prenatally anticonvulsant-exposed subjects had normal intellectual capacity but a small but significant group intellectually functioned at a subnormal level.
3) The smaller OFC did not seem related to cognitive functioning in adulthood.
4) Prenatal exposure to PB and PHT seems to induce minor genital anomalies and may affect reproductive behavior.
5) The gender dysphoria and extreme cross-gender behavior in some subjects may have been the result of their prenatal exposure to PB and PHT but that an environmental influence cannot be ruled out.

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Our findings that only a minority of anticonvulsant-exposed subjects are affected, corroborates the findings in other studies (Delassio, 1985). These results, therefore, support the hypothesis that these drugs are only harmful in foetuses susceptible to this type of teratogenicity (Buehler et al., 1990).

3.1.4. Discussion[19]

Dr Battino reminded the participants that intrauterine growth delay has been associated with maternal exposure to AEDs in some studies, and denied in others.

Head circumference, body weight and body length were all found to be affected by some authors, whereas for others only head circumference was influenced, being smaller in the offspring of epileptic mothers, regardless of AED therapy taken. In a few cases small head circumference in association with low body weight was observed, and in others short body length only was described. It was also shown that small head circumference solely was related to specific AEDs, namely PB and CBZ, or the two in association, and primidone. From the analyses carried out, it emerged that such risks tended to increase with the number of drugs used.

As for teratogenesis, the discrepancy of literature data can be partially explained by differences in study designs, mainly between retrospective and prospective studies. In particular, in the group with increased rate of foetal growth delay more prospective studies were needed. Also, variations in populations studied played a major role in this connection: populations were exposed to any AEDs, to monotherapies only or to individual AEDs in monotherapy or polytherapy.

Another main concern is the small number of pregnancies evaluated (from 14 to 963), with the exception of Bjerkedal's retrospective study which included more than 3000 cases, and a few others numbering more than 500 (Bjerkedal 1982; Bertollini et al., 1987; Battino et al., 1999; Wide et al., 2000a,b).

The variability of data analysis is another relevant factor. Only some papers reported data expressed as frequency of low birth weight or small head circumference, in comparison with control populations or national standards. The majority used international standards as comparison, or expressed data as absolute values, with and often without corrections for gestational age.

To conclude, there seem to be an urgent need for larger and better designed studies.

When discussing all the presentations looking at psychomotor development there was a general view that when carrying out any studies looking at the potential effects of AEDs it is important that all variables are taken into consideration, for example maternal seizure type and severity, maternal education level, parental head size. Only then can we try to start isolating factors that have an independent effect on the child. Dr Gaily commented on a problem seen in a previous study she was involved in, where the head circumference of children exposed to AEDs in utero was small at birth and also at follow up, but when they looked at the fathers of this group they also had significantly smaller head circumference than the fathers of the control group. Dr Battino agreed that this was something she also found in her clinical studies. Dr Tomson examined the problem that when looking at previously published studies, which examined the long term effects of AED exposure in utero, it is important to remember that these studies may have been conducted on patients who were exposed to different drugs regimes and the data will not necessarily be relevant to current clinical practices. Professor Lindhout commented that although this was certainly the case, lessons can still be learnt.

Dr Clayton-Smith raised the point that it is also vital that there is consistency between studies when recording and classifying malformations, to allow for comparisons to be made. In particular, the studies by Dr Dessens classify undescended testes as a malformation, whereas in a number of other studies this is not included. Dr Dean asked about

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the potential mechanism behind the gender dysphoria as this was something that had been seen in his study. Dr Dessens commented that they started looking at this feature due to the results seen in animal studies which showed changes in sexual dysmorphic behavior, and the explanation is that PHB and PHT disturb the foetal hormone balance which then effects the development of the foetal brain.

Dr Duncan commented that one of the problems with regards to gender dysphoria is that we do not know the rates of this condition in the general population, and it is also know to be a manifestation of depression. She added that you have to be very careful when interpreting these results. Dr Dessens replied that they had asked their patients to fill in an extensive questionnaire to take into account psychiatric factors, but agreed that you have to take into account all these potential influences.

3.2. Methods to assess psychomotor development[20]

Assessing the possible behavioral teratogenic effects of maternal use of antiepileptics during pregnancy requires long-term follow-up in infants. If such follow-up uses developmental tests, i.e. formal psychometric assessments of psychomotor development some specific demands must be outlined:

- The tests must be adequate for assessing development in the youngest age ranges.
- The test must be adequate for long-term follow-up over large age-ranges. This criterion follows the observation that sometimes developmental delays occur at young age that do not necessarily persist at higher age ranges. Conversely, some of the developmental delays are only detectable at higher age ranges. A continuum of assessment throughout childhood must, therefore, be possible.
- Developmental delay can be overall, resulting in a lower mental age, but may also result in delay in specific functions such as language delay. The tests must, therefore, cover a broad range of functions.
- If the test is to be used in an international trial, the test must be accepted internationally and norms must be available for different language areas.

A systematic review was undertaken using search programs of DIMDI and Medline for long-term follow-up studies in infants in the relatively recent period (1995–2002). The resulting studies covered several areas. Examples are:

- Behavioral teratogenic studies, e.g. after maternal use of benzodiazepine (Dolk and McElhatton, 2002) or of psychiatric drugs (Arnon et al., 2000) or of alcohol/drugs or of antiepileptics (Dean et al., 2002).
- Neurodevelopmental outcome with children undergoing heart transplantation or renal transplantation at young age (Baum et al., 1993).
- Neurodevelopmental outcome in low birth weight infants or preterm infants (Grunau et al., 2002; Sommerfelt et al., 2002).
- Long-term outcome of PB treatment in toddlers (Shankaran et al., 2002).
- Long-term outcome of infants chronically exposed to neurtoxicants (Grandjean et al., 2001).
- Long-term outcome after perinatal or neonatal infarction (Govaert et al., 1999) or hypoxic ischaemic encephalopathy (Aggarwal et al., 1998) or asphyxia (Maneru et al., 2001).
- Long-term follow-up developmental studies in chronically ill children (Gorman et al., 2001; Lejarraga et al., 2002).

In total 283 studies were identified. The Bayley Scales of Infant development (Bayley, 1969) was the most commonly used test for psychomotor development and was used in 63% of these studies. All other tests were only mentioned in two or three

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studies. The Bayley Scales of Infant development ('Bayley') belongs to the class of developmental tests that have some characteristics in common:

- Developmental tests have mental age as outcome not IQ. For example 'a child of 4 years has a mental age of 2 years'.
- The tests assess samples of behavior, representative for each age. The tests, therefore, measure these samples for each age-level until the individual child's ceiling level is achieved.
- These tests are used to assess development, i.e. to perform long-term follow-up.

Using the previous criteria, two developmental tests are available:

- Bayley Scales of Infant Development: Age range 2 months up to 21/2 years (Bayley, 1969). The test has mental age as outcome and two subscales: mental and motor development
- McCarthy Scales of Children's Abilities: Age range 21/2 years up to 81/2 years (McCarthy et al., 1972). The test also has mental age as outcome but also provides a profile for cognitive development.

Additionally, the Denver Developmental Screening Test is available. This test has an age range of 0–6 years but is, in contrast with the Bayley and the McCarthy, a screening test (Frankenburg et al., 1970). The test uses mental age as outcome and gives a developmental profile. The Bayley and McCarthy tests are formal assessment methods, which require specific training to use the tests. The Denver is a combined interview and assessment methods.

For use in a study on behavioral teratogenic effects of antiepileptics, it is proposed that the following battery of tests to assess psychomotor development:

1) Before 6 months: The Denver developmental screening test. Outcome: discrepancies in weeks per area.
2) Start formal assessment at 6 months with: Bayley Scales of Infant Development: Age range 2 months up to 21/2 years (Bayley, 1969). Outcomes: mental age and separate scores for mental and psychomotor development.
3) From 21/2 years up to about 6–8 years: McCarthy Scales of Children's Abilities: Age range 21/2 years up to 81/2 years (McCarthy et al., 1972). Outcomes: mental age and sub scores for separate cognitive functions.
4) From about 6 years: Wechsler intelligence and school performance (WRAT) plus social behavior and personality development.

3.2.1. *Discussion*[21]

Dr Vinten agreed that there is a need for a recognized comprehensive battery of tests to be applied in order to assess the possible teratogenic effects of intrauterine AED exposure on children born to women with epilepsy. To achieve a valid assessment tests need to be able to:

1) Assess different facets of development in order to identify specific deficits in neurodevelopmental. Previous research has reported that AEDs are associated with specific cognitive functioning recent research has suggested AEDs may have a detrimental effect on language functioning.
2) Demonstrate evidence of reliability and validity.
3) Must be sensitive to detect change over time for use in long-term follow up studies.

A growing number of both retrospective and prospective studies have looked at psychomotor development in children exposed to AEDs in utero with conflicting results. Some have reported a high prevalence of developmental delay, compared with controls (Hill et al., 1982; Gaily et al., 1988; Hanson et al., 1976; Rovet et al., 1995) others have reported a transient impairment (Normura et al., 1984) and other studies have reported no deficit (Nelson and Ellenberg, 1982; Shapiro et al., 1976; Table 3).

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Table 3
Reported effects of exposure to AEDs in utero

Author	Age	Test	Results
Retrospective			
Spiedel and Meadow, 1972	0–12 months	None	1.5% Development Delay
Huth et al., 1982	3–9 years	ITPA/CMMS	No Change in result
Dessens, 2000	16+	WAIS (subtests)	Decrease in IQ
Moore et al., 2000	3–16 years	Questionnaire	Increase in impairment
Adab, 2001	4–18 years	Questionnaire	Increase in S.E.N
Williams et al., 2001	3–9 years	Bayleys/WISC	Decrease in scores
Prospective			
Hill et al., 1974	18–21 months	Gesell	Decrease in scores
Shapiro et al., 1976	8 months, 4 years	Bayleys/WISC	No Change reported
Hanson et al., 1976	7 years	WISC	Decrease in IQ scores
Gaily et al., 1988	5.5 years	WPPSI	Decrease in IQ scores
Jones et al., 1989	6 months, 2.5 years	Bayley\WPPSI	20% Developmental Delay
D'Souza et al., 1990	2.5–3.5 years	Griffiths	No Change reported
Van der Pol et al., 1991	6–13 years	Dutch Test R.R.S	No Change reported
Leavitt et al., 1992	12 months	Bayleys	No Change reported
Rovet et al., 1995	7 months–7 years	Bayleys/McCarthy	Decrease in language skills
Koch et al., 1999	11–18 years	WISC/WAIS	Decrease in IQ scores
Wide et al., 2000	9 months	Griffiths	No Change reported

Due to the wide variation of tests that have been applied and the failure of a standardized approach it has been difficult to draw any meaningful conclusions regarding developmental outcomes and the degree of risk associated with intrauterine AED exposure, especially for specific drugs. In order to conduct a comprehensive assessment confounding factors such as the maternal and paternal intelligence should be evaluated. The authors strongly recommend a uniform approach to assess the neurodevelopmental effects of AED treatment.

In the general discussion, Dr Cross raised the issue that as we become aware of the prevalence of verbal deficits in the AED exposed children it may be necessary to expand our current methods to look more specifically at speech and language components. Dr Gaily in particular elaborated on a method of assessment her group uses, which is particularly good for testing linguistic ability. The group uses a neuropsychological test battery which has been validated in both the USA and Finland. The test looks qualitatively at the basic skills for linguistic performance and development, and has various visual subtests. Dr Gaily felt that, using this test, it may be possible to find the basic dysfunctions underlying the verbal delay in these children.

Professor Chadwick queried if these early developmental tests can predict adult IQ levels. In reply Professor Meador commented that there were several measures that correlate with adult IQ but these are all for children over the age of 6 years, and recommended that children are followed to at least six and if possible for even longer. Dr Battino also commented on observations from the Milan study, which had found children who had a low IQ at 3 years but had a normal IQ when assessed at 6 years. Dr Gaily felt that if we were just looking to get an overall assessment of mental deficiency then this could be picked up at around 3 years, but agreed that when looking at specific cognitive deficits the tests should be carried out at around 5–6 years. Another problem raised by Professor

Chadwick was that it is very difficult to keep contact with these families to allow for long term follow up studies.

4. Prospective studies

The data available from published studies of the outcome of pregnancy in WWE come from many different groups with differing protocols for collecting and analyzing data. Many of these studies were also from small groups making statistical analysis difficult and tended to have been done many years ago so a number of the most recent drugs to come on the market have not been analyzed. The differing protocols make it difficult for a physician to give an expectant mother the correct information. This has highlighted the importance of worldwide registers where the data is analyzed by a set protocol and allows for better exchange of information.

A number of questions are still to be answered namely:

1) the relative risk associated with each drug;
2) the benefits of monotherapy or low dose schemes;
3) the incidence of minor malformations;
4) the fraction of the risk attributable to the drugs and the fraction attributable to the underlying disease;
5) the long term effects of the AED's on the child.

For all these reasons, pregnancy registers have been set up in a number of countries world wide by both research teams and drug companies. The main aim of these registers is to enrol a large number of women who have taken AED's during pregnancy and to ensure long term monitoring of the children. These registers also allow for a better standardizing of how the malformations are measured and recorded.

In the last decade pregnancy registers have been set up in Europe (EURAP), North America, Australia and India, although recently the Australian and Indian groups have merged with the European groups. With the data acquired through these registers it will be possible to provide better counseling for an epileptic woman who is considering having a child.

4.1. Lessons to be learned from published studies[22]

To evaluate the reliability of published research on teratogenesis induced by AEDs, about 700 papers were extracted from Medline, using a broad strategy search. AED teratogenesis has been studied worldwide and for a long time, as the first studies appeared during the 30s.

Despite the efforts made in so many countries for so many years, the results achieved so far are poorly homogeneous and conflicting.

Of the 236 cohort studies retrieved, 93 (total of 24 036 cases) were selected and reviewed.

Selection criteria were:

1) language of publication (English, French, Italian);
2) originality: when more than one paper was published on the same cohort, only the latest was considered;
3) comparability: studies focusing on particular aspects, i.e. a specific malformation, were excluded;
4) source availability: papers published by national Journals were mostly unavailable.

Analysis of these publications has shown a high variability among reported malformation rates: 0–42.9% ($7.7 \pm 5.9\%$) all pregnancies; 0–13.6% ($3.0 \pm 4.2\%$) untreated; 0–47% ($8.9 \pm 6.6\%$) treated; 0–42.9% ($8.4 \pm 8.3\%$) monotherapies; 0–55.6% ($10.7 \pm 10.1\%$) polytherapies. Similar differences were observed when AEDs on monotherapy were examined separately: 0–50% PHT and PB; 0–25% CBZ; 0–33% valproic acid; 0–3.8% primidone. Of note, each major AED was, at least once, found to be the most teratogenic.

[22] Dr Dina Battino, Neurological Institute Carlo Besta, Via Celonia, 201 33, Milan, Italy.

The discrepancy of literature data mirrors the great variability of study designs, tested populations and classification criteria of malformations, which in turn is due to the specificity and also complexity of methodological issues involved in the investigation of AED teratogenesis (Robert, 1992; Irl and Hasford, 2000; Battino, 2001; Dolk and McElhatton, 2002).

The studies were either prospective or retrospective, with or without matched controls, unmatched or with non-epileptic treated controls. Some gathered data from the general population. Few were matched for all variables, such as maternal age, social status and above all genetic background, factors known to affect the teratogenic endpoints. Study duration differed greatly, from just a few months to several years (the longest took 37 years). The same held true for follow-ups (1 day to several years).

In addition, most of the studies provided poor information on potential risk factors, such as other diseases or exposures, time of exposure, maternal age, social status, epilepsy duration or severity, occurrence of seizures and family history of malformations. Limited information was also available concerning drop-outs and patients' compliance.

Populations examined were also diverse: in some cases only pregnancies of AED-exposed epileptic women were included, whereas others considered treatment-free epileptic subjects, or non-epileptic women taking AEDs for other reasons.

Another main point is that authors adopted different exclusion criteria, such as maternal congenital malformations, other diseases or exposures, multiple pregnancies of the same patient. Moreover, malformation rates were calculated in populations including live births only or live births and therapeutic abortions and/or stillbirths and/or neonatal deaths and/or drop-outs.

Regarding teratogenic endpoints all studies considered major malformations, except for some which included also minor anomalies or intrauterine growth delays, as well as conditions like chromosome abnormalities, inguinal and umbilical hernias, hip dislocation, first degree hypospadias, hydrocele, torticollis, microcephaly, postural deformities and ptosis. A lack of standardized definitions for malformation criteria has emerged too: many papers did not provide malformation definitions, some reported personal definitions, and a few used known classifications like ICD8-ICD10.

Methodology and timing of malformation assessment were often different. While some studies included malformations described in medical records or seen by physicians, others used rigorous and standardized physical examinations. As for the timing of assessment, it was carried out either prenatally or postnatally. Thus, information, which was not readily apparent at birth was sometimes lost or, on the contrary, malformations which disappeared soon after birth were included. However, the main limitation of available studies is probably the small number of pregnancies included; few numbered more than 500 pregnancies, and the majority had fewer than 150.

Finally, older studies were characterized by poor quality control or long intervals between data collection and processing, thus bringing about low accuracy and high drop-out rates.

In the general discussion Professor Wells raised the point that when talking about drugs that are converted into a reactive intermediate, it would be expected that even at therapeutic concentrations some people would be more sensitive. In designing a prospective study to identify which of the subsets of children will be at particular risk, researchers should be encouraged to look at molecular end points in the embryo. Dr Battino replied that the studies must have a balance between the need to collect all of information, and the need to be able to recruit enough pregnancies to make statistically valid conclusions.

Professor Lindhout commented on the potential benefits of collecting samples during pregnancy that can be used later when looking at metabolic profiles and genetic susceptibility. Professor Chadwick asked about the power to detect associations between outcomes and relatively common polymorphisms, and the problems of multiple testing. Professor Lindhout agreed that power was a very important issue, and suggested that a means of doing this would be to start with candidate genes and see if there is an association. For example, in investigating how folate metabolism may play a

role in NTDs, knowing that one of the 150 genes that are involved in folic acid metabolism may play a role, screening for snips in those 150 genes to detect a signal in the population should be undertaken. He felt that studies carried out on animal models looking at gene expression in susceptible and resistant strains may help pinpoint a specific gene that can then be screened for in the human population.

4.2. NEAD study[23]

The Neurodevelopmental Effects of Antiepileptic Drugs (NEAD) study in the USA addresses an important health care issue for women and their children. The use of AEDs in women of childbearing age for epilepsy and for other indications (e.g. pain and psychiatric disorders) is common, but physicians need additional data, which are critical to adequately advise and direct treatment in these women (Meador, 2001). Animal studies have clearly demonstrated that several of the older commonly employed AEDs impair behavioral neurodevelopment at dosages less than those required for anatomical defects (Finnell and Dansky, 1991). Further, several recent studies suggest that AEDs may impair behavioral neurodevelopment in humans (Reinisch et al., 1995; Adab et al., 2001; Dean et al., 2002). The potential consequences of such cognitive and behavioral deficits in humans are severe in terms of both personal and societal costs.

However, controversy exists on the following issue to be addressed by this proposal. Do the most commonly used AEDs have differential effects on neurobehavioral outcomes in children exposed in utero? The primary objective of this study is to differentiate the relative risks/benefits of the four major AEDs used in the treatment of WWE in terms of their children's neurobehavioral development following in utero exposure. The study objectives will be addressed by a prospective, parallel-group, cohort observational design, multicenter investigation enrolling four groups of pregnant women on AED monotherapy (i.e. CBZ, lamotrigine, PHT, and valproate). These four AEDs are currently the most commonly used AEDs in pregnant women treated at tertiary medical centers in the USA.

The primary outcome variable is IQ score of the children. Other measures of neurobehavioral development will also be assessed. Mechanistic studies include homocysteine, G6PD, and genetic testing. Women are being enrolled during pregnancy to follow their children to 2–3 years old in the initial grant, but it is planned to ultimately follow the children to at least 6 years old. The children's' IQ scores will be covaried with the maternal IQ scores in the primary analysis. In addition, IQ scores will be obtained for the father and a primary relative of the mother to assess the inherited contribution from the father and to assure the contribution from the mother is not underestimated due to the effects of epilepsy and its treatments on her IQ. Multiple environmental factors are also being assessed. The results of the study will impact the clinical management of women receiving these medications, and improve the health of their children.

For more information on the NEAD study see the website at http://www.neadstudy.com.

In the general discussion concern was raised about the population studied, as there was a high percentage of women who were educated to the college level and this was though to be unrepresentative of the general population. Professor Lindhout suggested consulting a population geneticist as the children's IQ may regress towards the mean. Professor Meador commented that he is comparing the different drug groups directly, and if there is no difference in the maternal IQ between study groups then this would not be an issue. The study is not designed to answer whether or not AEDs per se decrease IQ levels, but only to look at the relative effects of the four different drugs.

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4.3. UK epilepsy and pregnancy register[24]

With the advent of a number of new AEDs in the 1990's and their increasing use by neurologists and others throughout the UK, there was concern as to the safety of these drugs in human pregnancy (Crawford et al., 1999). Animal studies suggesting that many of the drugs conveyed advantage were re-assuring to some physicians but not to all.

It would have been unethical to carry out a randomized controlled trial in women of child-bearing years contemplating pregnancy on anti-epileptic medication and, therefore, a prospective observational register was felt to be the most logical method of assessing the safety of new AEDs in pregnancy.

As pointed out by Br Battino above, previous studies of AED safety in pregnancy frequently contained a number of methodological flaws. Often they had come from specialized epilepsy centers, the majority were retrospective and usually contained inadequate numbers of patients on individual drugs in monotherapy to draw statistically sound conclusions.

The UK Epilepsy and Pregnancy Register was established in 1996. Initially it was helped by the British Neurological Surveillance Unit and subsequently by a grant from the Epilepsy Research Foundation. It has been widely supported by physicians, specialist nurses, midwives and patients alike. It has been widely advertised in the form of a poster campaign and also a website (http://www.epilepsyandpregnancy.co.uk) has been set up (Morrow et al., 2000).

The running of the register and the results are overseen on a semi-annual basis by a multi-disciplinary group. To date over 3000 women have been registered in the early stages of pregnancy. Registration has been kept relatively simple patients' demographic and other details including drug type and dose, epilepsy/seizure type and frequency and general practitioner details are noted at registration.

Three months after the estimated date of delivery, the general practitioner is contacted and the outcome of the pregnancy and any abnormalities or investigations are recorded. Any previous pregnancy details are also noted, as is a family history of any major anomaly. All registrants give informed consent and the study has been approved by every ethics committee throughout the United Kingdom.

The study has been powered to have 80% chance of picking up a doubling of abnormality rate with 300 patients on each drug in monotherapy. To date this target of 300 patients on monotherapy has been reached with CBZ, valproate and lamotrigine. There are a number of newer agents which are just recently starting to be used throughout human pregnancy and clearly it is important that the register maintains vigilance over these drugs (Craig et al., 1999; Morrow et al., 2000, 2001).

Although the results of the register are preliminary there have been a number of important issues raised already, and the study is starting to produce statistics that could be useful to clinicians, (full data in Morrow et al., 2002) The study has shown that the crude MCM rate for monotherapy is around 4% (3.2–5.3%) and for polytherapy 6.3% (4.3–9.1%). CBZ monotherapy is seen to carry a 2.3% (1.4–4%) risk of a MCM, with lamotrigine having a 3% (1.5–5.7%) risk and PHT monotherapy having a 3.4% (1–11.7%) risk. The relatively low risk associated with PHT is surprising considering that this has been considered a particularly dirty drug. One explanation for this could be the change in prescribing practices since many of these initial studies were done. Sodium valproate was seen to have the highest risk factor at 7.2% (5.2–10%), although there did not seem to be a dose response.

One result which could be of particular clinical importance, although the number of cases looked at so far is very low (64), is the risk associated with valproate and lamotrigine in combination. The risk of a MCM in this group appears to be around 11.9% (5.9–22.5%). This is noteworthy as this

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combination is widely used in the UK due to its efficacy, and we may need to think again about prescribing these AED together is this group.

Further studies are ongoing in particular the follow-up of children born to mothers taking anticonvulsant medication are being assessed in a cohort study.

In the general discussion Professor Wells raised a question about the lack of dose response, and commented that the dose level may not be a particularly good way to assess risk if genetic predisposition is to be detected. Professor Lindhout remarked that in two studies carried out in The Netherlands a dose response was seen with valproate, and this may be attributable to peak dose rather than total daily dose. Dr Morrow replied that they had taken this into account by looking at the slow release formulations and found no difference, but these were preliminary results. Dr Clayton-Smith commented on a study that she had been involved with, where a mother had two children while she was taking low dose of valproate and both these children had anomalies, and she felt this further supported the hypothesis that there are genetically susceptible people for whom the risk is greater even at low doses.

Professor Perucca asked about the results concerning lamotrigine. Dr Morrow's study has shown that the risks associated with lamotrigine, while not being higher than CBZ, were not significantly better. This was unexpected as it was hoped that this newer drug would be safer for pregnant woman. Dr Morrow replied that the GSK study, which is due to be published soon will report the same malformation rates. Again the issue was raised about the problem of pregnancies where the outcome is known. This was seem to be a difficult issue and the participants were not sure of how to approach this problem.

4.4. EURAP[25]

In view of the inadequate sample size and other methodological shortcomings in previous studies (Beghi and Annegers, 2001), a prospective international AEDs and pregnancy registry (EURAP) was set up in Europe and later extended to several other countries (Tomson et al., 2001). The primary objective of EURAP is to compare the teratogenic potential of different AEDs and the primary teratogenic endpoint is the presence or absence of major birth defects.

Women taking AEDs for any indication at the time of conception are eligible for inclusion, subject to their willingness to provide informed consent. To avoid selection bias, only pregnancies registered before foetal outcome is known and within week 16 of gestation contribute to the prospective study. Retrospective cases are also collected as they may provide signals, but are not included in the evaluation of comparative risk. Information on patients' demographics, underlying disease, seizure frequency, family history of malformations, drug therapy and a set of other potential risk factors is obtained, and follow-up data are collected once at each trimester, at birth and at 1 year after delivery. Data are collected online, and feedback between the central registry and reporting physicians or national co-ordinators allows rapid collection of missing data and correction of inconsistencies. Foetal outcome is recorded descriptively, and a central committee that is unaware of the type of drug exposure classifies and encodes the malformations.

The enrolment rate to EURAP has increased gradually and now exceeds 100 new pregnancies per month. As of August 2002, 269 collaborators in 25 countries from four continents (Europe, Asia, Australia and South America) have enrolled 1725 pregnancies with complete and corrected case report forms. Of these, 1270 (74%) are prospective. Nine hundred and fifty two of the prospective cases have been followed until 3 months after delivery whereas 457 had completed the 1-year follow-up after birth. Seventy six percent of the prospective cases were on monotherapy with CBZ as the most frequently used AED followed by valproic acid, lamotrigine and PB. There were 16

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stillbirths, nine perinatal deaths, and 38 induced and 71 spontaneous abortions among the prospective cases. So far a total of 44 cases with major congenital malformations have been identified in the prospective cohort, representing a malformation rate of 5%. Five of the 44 malformations were detected among those with induced abortions or stillbirths. The risk of birth defects in relation to individual AEDs will be analyzed only when the number of pregnancies that have been followed is sufficient for a meaningful statistical analysis.

Although the primary objective of the core EURAP protocol is to study the risk of major birth defects, extension protocols are being developed to address other related issues. Hence, a protocol on the pharmacokinetics of new AEDs during pregnancy and the perinatal period has been implemented and protocols for follow-up of long-term postnatal development and pharrnacogenomics are under consideration.

In the general discussion Dr Creasy asked about the different reporting protocols between EURAP where it is a professional register and the NEAD study where it is a personal register. Dr Tomson felt that the personal registers may present a selection bias of women who feel they are more at risk.

4.5. Power considerations: will these studies answer all of our questions[26]

Company driven (Reiff-Eldridge et al., 2000) and independent epilepsy and pregnancy registries (North American Pregnancy and Epilepsy Registry, 1998; Beghi and Annegers, 2001; Tomson et al., 2001) have been established in recent years. While the value of the individual company registries is limited by the lack of controls or comparator, the establishment of collaborative registries to assess outcome in the offspring of mothers treated with AEDs is a huge step forward in the efforts to obtain reliable data on comparative risks associated with different therapies. It is expected that these studies will eventually lead to more rational, evidence-based pre-pregnancy counseling.

Will these studies answer all our questions? The easy answer is no, and we can also expect that the findings will raise additional questions that we can hardly anticipate today. Several scenarios can be envisaged. If, as in previous studies, the enrolment of pregnancies is slow, some treatments may be outdated for other reasons before their relative safety can be adequately assessed. However, the most disturbing scenario is the situation where two major independent registries arrive at conflicting results: while it is hoped that this will not materialize, such a scenario will lead to a difficult search for the identification of factors responsible for any discrepancy. Despite the huge number of patients being evaluated by the registries, subgroups exposed to less frequently used AEDs (and, most importantly, specific AED combinations) will remain relatively small: at best, analysis for these data sets will only be useful to generate hypothesis, which will need to be tested in further studies. Similar considerations will apply to evaluation of the influence of other risk factors such as dosage (at least for some drugs), dosing rates, serum drug levels, seizures, diet, potential protectants (e.g. folic acid), other environmental factors, and genetic background. Within the EURAP collaboration, extension protocols have been (or are being) designed to address prospectively additional aspects such as changes in the disposition of new AEDs during pregnancy and in the newborn, the impact of breastfeeding, mechanisms of teratogenesis (including pharmacogenomic influences) and long-term postnatal development. Whereas the identification of major birth defects, the primary end-point of most registries, may appear to be straight forward, the assessment of postnatal neurodevelopment is more complicated. An adequate evaluation of such development is much more demanding with respect to follow-up time, cooperation of the child and its family, and resources necessary for assessment. Optimal tim-

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ing and the most appropriate and cost-effective investigational methods need to be determined to permit evaluation of sufficient numbers of children. One major benefit from registries is that they enhance collaboration among scientists interested in disparate aspects, provide cross-fertilization for additional collaborative projects in areas not addressed by the core protocols.

5. General discussants

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